PLANT PHYSIOLOGY

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Page 61, line 8 from bottom, dele period and word "then," and place remainder of sentence in parentheses.
Page 76, table XIV, bottom of column 4, for "0.372" read 0.0372.
Page 78, line 16, for "6.67" read 5.56.
Page 100, fig. 7 replaced by cut provided with April number.
Page 115, lines 12 and 13, transpose $Cl^{[-]h} = 158$ and $K^{[+]h} = 94$.
Page 128, line 12, for "114" read 14.
Page 179, table I, transpose position of "Alpha carotene" and "Beta carotene" in first column.
First cover page, April number, line 15 table of contents, for "SWEET" read STREET.
First cover page, July number, line 8 table of contents, for "452" read 453.
Fourth cover page, July number, lines 7 and 8, for "University of Michigan" read Cornell University.

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PLANT PHYSIOLOGY

JANUARY, 1934

RELATIONS OF GERMINATING SOY BEANS TO TEMPERATURE AND LENGTH OF INCUBATION TIME[1]

T. I. EDWARDS

(WITH SIX FIGURES)

Introduction

Because much physiological study deals with plants that are secured from seeds, the conditions that prevail during the preliminary germination period constitute a very important part of such studies. Individual variability among the similar plants of an experimental series makes physiological experimentation very laborious, and presents one of the most difficult problems with respect to both technique and interpretation. Many recent experimenters have attempted to reduce variability through some sort of plant selection, and such selection usually begins with the seeds employed, —as when the seeds are chosen according to their genetic background and with reference to size, weight, color, etc. Young seedlings, just after germination, are commonly subjected to more or less rigorous selection according to size and general appearance, but especially according to apparent vigor. Such selection tends to restrict variability and furnishes experimental series of plants that are more nearly alike in their intrinsic physiological nature, as well as in their variability, than would be the case without selection. When young seedlings are selected for uniformity, the standard set of conditions that prevailed while germination was in progress becomes a basis for the selection. Consequently any advance in our knowledge of how specified lots of seed may be influenced in their germination performance by the environmental complex prevailing during the period of germination should be useful in the preparation of seedlings for experiments dealing with more advanced developmental phases. In this connection see GOODSPEED (**11**), CORRENS (**5**), HAASIS (**12**), and TANG (**36**). Furthermore, a number of students (**20**) have presented evidence leading to the

[1] Botanical contribution from the Johns Hopkins University, no. 118.

supposition that the environmental conditions prevailing during seed germination may exert a persistent influence on the vigor and capacity for response of the resulting plant; in other words, conditions that were effective during seed germination may limit subsequent growth and yield. From these and other considerations it is evident that the subject of seed germination is an important one, not only in itself but also as fundamental to the study of more mature plants.

From the standpoint of experimental technique, studies on seed germination are in many ways much less difficult than studies dealing with more mature phases of growth and development. In many instances germination may be studied in the absence of illumination, with the avoidance of many difficult and complicated considerations that inevitably present themselves when cultures are carried out in light; for light intensity, light quality, and light fluctuation may all be left out of consideration in experiments on germination of many common sorts of seed, since ordinary seeds generally carry sufficient nutrient material to support germination and the early stages of seedling development. Although it is desirable to employ as germination medium a weak solution of inorganic salts rather than distilled water, yet excellent germination may be secured with a great variety of different solutions (**10**).

The experiments with which this paper deals show, for a specified lot of Black Eyebrow soy bean seeds, the influence of maintained temperature on the proportion of seeds germinating with incubation periods of different lengths. For a number of different combinations of temperature and time the resulting percentage values were studied by simple statistical methods, to bring out some characteristics of the variability of the lot of seed used. For each maintained temperature tested, increments of germination percentage for successive time intervals were studied.

Maintained temperature and time were the only experimental variables involved. All other influential conditions were alike for all tests, or else their complex differed from test to test only as they may have been influenced by temperature and time. Of the various influential conditions that may be varied in an experimental study, maintained temperature is especially suited to experimentation and analysis because it acts directly upon the seed protoplasm and is not subject to modification by integuments or protoplasmic membranes. For these tests temperature was an independent variable, depending only on the environment and not being significantly influenced either by the nature of the seeds or by their activity. The duration factor, time, is naturally an unavoidable component of any experimental procedure, and the length of exposure to a given set of conditions has a powerful influence on the organism's responses.

Soy bean (*Soja max* Piper or *Glycine hispida* (Moench) Maximowicz) seed was chosen as the subject of this study for several reasons. Soy bean is an important crop plant in many regions, and it has been used in a number of physiological studies as well as in field experimentation. The seeds are of convenient size for such experiments, and they germinate promptly in darkness when other conditions are suitable. A commonly cultivated, natural hybridization between different varieties of this species appears to be uncommon (**8**) and homozygous lots of seed are consequently obtainable.

A general review of the literature on temperature relations and time relations of germinating seeds is already available (**6**), and only the publications that bear directly on this study will be mentioned here. Several studies on the germination of soy bean seeds and the early growth of seedlings have been reported recently. WILSON (**40**) and OATHOUT (**28**) studied the general temperature relations of germinating soy bean seed. Employing microchemical methods, VON OHLEN (**38**) traced the major metabolic changes of early stages of development of Manchu soy beans. HAFENRICHTER (**15**) and STARK (**35**), using the same stock of seed, investigated CO_2 output and change in amino-acid content in seedlings maintained at different temperatures. JONES and TISDALE (**19**) cultured soy bean plants in pots of soil maintained at different temperatures between 12° and 40°, while the shoots were all exposed to approximately similar greenhouse conditions, the air temperature being 14°–18° for all cultures. These plants tolerated soil temperatures between 12° and 36° but failed to grow when the temperature of the soil was 40°. Root development was about the same for all soil temperatures tested above 18°. The greatest dry weight of tops was secured with soil temperatures between about 24° and about 35°. Nodule development, reckoned on the basis of dry weight, was most vigorous at a soil temperature of 24°. On the basis of JONES and TISDALE'S tests, the soy bean plant appears to be well adapted to a range of soil temperatures much broader than the range tolerated by many other crop plants that have received attention in this respect.

Experimental methods and primary numerical results

With the exception of the maintained temperatures, a uniform procedure was followed for all the experiments. The seeds lay on agar plates in covered cylindrical petri dishes 10 cm. in diameter and 1.5 cm. deep. Each experiment embraced four sets of five dishes, and each dish had 20 seeds.

The battery of maintained-temperature chambers of this laboratory (**23**), which has served for many earlier studies, was employed, but only five of the seven chambers were actually used. The automatic controls

were set so as to provide the following five temperatures: 24.5°, 28.5°, 33.0°, 36.5°, and 40.0°. The two unused chambers had temperatures of 20° and 17°. The range of temperature fluctuation for any of the used chambers was about ± 0.5°.

In this apparatus the seven chambers are in a linear series, each provided with a continuously stirred water-jacket. Each water mass is separated from the two adjacent ones by means of an uninsulated sheet-iron partition, and the entire series is inclosed in a light-proof cabinet with hair-insulated walls and bottom. Access to the chambers is had from above, by means of removable lids with cork and wood insulation. Heat is supplied at one end of the series from a thermostatically controlled tank of hot water and it is removed at the other end by means of another tank of water, the latter being thermostatically controlled by means of a mechanical refrigeration machine. The chambers are vertical cylinders (38 cm. in diameter and 38 cm. deep), and all parts of each chamber remain at practically the same temperature, but the temperature of each chamber differs from the temperature of adjacent chambers to a degree determined by the settings of the two thermostats. In each chamber the petri-dish cultures were stacked loosely, in horizontal position, in a suspended basket of perforated sheet metal.

The seeds used were all from a single lot of Black Eyebrow soy bean seed grown in Indiana in 1930, supplied by the Eastern States Farmers Exchange through the courtesy of Mr. C. W. Clemmer, of Springfield, Mass. It was received in December, 1930. These seeds were characterized by a black hilum and by a black area on each side of the hilum, the rest of the seed surface being olive green or brown. The seed-coat markings corresponded to the description given by MORSE (**26**) for seeds of this variety, and the seeds of this lot were very similar to those of other lots of the same variety obtained from other sources, although those of the lot used were a little smaller on the average; 100 of them weighed 14.1 gm. There was considerable variation in the relative sizes of the areas occupied by the two colors but no obvious relations were detected between seed-coat markings and germination behavior. The whole stock of seeds was sorted at the beginning of the study and a small proportion of obviously imperfect or notably unusual individuals was discarded. The remaining supply was stored in a cloth bag in a loosely covered metal can kept in a basement room. OATHOUT (**28**) found such treatment very satisfactory in preserving the vitality of his soy bean seed. The experiments were performed between February 6 and March 22, 1931, so that none of the seeds used differed in age by more than a month and a half.

The medium for these tests was an agar gel containing per liter: 5.0 gm. of the Digestive Ferment Company's "Bacto-agar," 0.0018 mol. (0.2451

gm.) of KH_2PO_4, 0.00052 mol. (0.1228 gm.) of $Ca(NO_3)_2 \cdot 4H_2O$, and 0.0015 mol. (0.3707 gm.) of $MgSO_4 \cdot 7H_2O$. The salts were used because a dilute solution had been found by many workers to be more satisfactory than distilled water as a medium for seed germination. The solutes used in this way have in many instances been considered as "seed stimulants," and a great number of organic as well as inorganic substances have been assigned to this category by various investigators; NIETHAMMER (**27**) has published an extensive summary concerning seed stimulation. When seeds are germinated (and even when just preliminarily soaked) in distilled water, it is always possible that substances emanating from the seeds or from accompanying microorganisms may so modify the adjacent medium as to affect germination, and it appears that such effects are not so apt to occur when a dilute salt solution is used instead of water. Loss of essential ions from the swelling and germinating seeds appears to be retarded by the presence in the medium of suitable salts at suitable concentrations. The small traces of injurious substances that are apt to be present in distilled water seem generally to be robbed of their toxicity when nutrient salts are also present at low but considerable concentrations. GERICKE'S (**10**) experiments on germinating wheat seed showed no consistent differences in the physiological effects of dilute 3-salt solutions having very different partial concentrations of the salts. His solutions were all markedly better than distilled water.

The three salts were first dissolved in distilled water from a Barnstead still, to make SHIVE'S (**33**) solution R5C2 (0.175 atm.). (This low total concentration is the same as was used by GERICKE, being only one-tenth as great as the lowest total concentration used in SHIVE'S series of experiments.) To 2.5 liters of the 3-salt solution thus prepared (the pH value of which was found to be 4.3), 12.5 gm. of "Bacto-agar" were added and the mixture heated in a beaker over a boiling water-bath until all granules had disappeared. Then distilled water was added to make the volume up to the original one, 2.5 liters. Each stock of gel thus prepared was stored in stoppered flasks at 2°–3°. Storage was never for more than five days, in which time no apparent alteration of the gel occurred. Its pH value was 5.6, as ascertained by the use of a quinhydrone electrode as well as by colorimetric means.

A few hours before a series of cultures was to be started, a flask of gel was removed from the refrigerator and heated in a water-bath until liquified. Then 25 ml. were pipetted into each of 20 heat-sterilized petri dishes, which were immediately placed on a previously cooled slate slab, where the agar plates rapidly solidified. This rapid cooling saved some time and lessened the amount of water that condensed on the lids of the dishes during the cooling process.

Twenty seeds, taken at random from the stock, were about equally spaced on each of the 20 plates, each seed lying on its side. The seeds sank slightly into the agar gel, and at the end of 12 hours about one-third of the seed volume was below the gel surface, the hilum being just above the surface. Thus a large area of the testa was in contact with the gel, which favored water absorption while gaseous exchange between seed and atmosphere was not greatly retarded. Rapid absorption of water took place, showing that the seed testas were readily permeable. About 25 minutes was required to distribute 400 soy beans on 20 agar plates.

It will be noted that no preliminary sterilization treatment was applied. While such treatments undoubtedly may be effective to prevent or retard subsequent development of microorganisms in the cultures, yet they are apt to exert some influence on germination. As has been remarked, a great variety of chemical substances have been observed to act as seed stimulants, and consequently it seems unlikely that any soaking treatment drastic enough to kill microbial spores would leave the seed embryo unaltered, aside from the effect of water absorption during the treatment (**9**). Preliminary soaking, which usually accompanies sterilization treatment, may in itself exert some influence on subsequent germination performance (**20**). It was found, for example, that Manchu soy beans that had received a preliminary soaking of one hour in water or in dilute solutions of ethyl alcohol required less time for germination on agar plates than was required when similar seeds had been placed on the plates without preliminary soaking. Many of these seeds were obviously injured in a single hour of soaking, however; in many instances the cotyledons were cracked or broken loose from the embryo or the testa was torn. Also the soaked seeds showed greater variability in the time required for germination than was shown by seeds laid on the agar dry. Since preliminary soaking generally hastens subsequent germination, it might be employed in studies on seed of Black Eyebrow or other varieties of soy bean if suitable time and temperature conditions were established, and if a suitable standard solution were used.

Immediately after placing the seeds, the 20 cultures of a series were installed in the requisite temperature chambers where they remained from 24 to 28 hours, except that they were removed for observation at 2-hour intervals after germinated seeds began to appear. Preliminary experiments showed when germination was to be expected at the several temperatures, and so the cultures were usually not removed for examination before the end of the tenth or twelfth hour of incubation. A culture was never out of its chamber for more than four or five minutes at any observation. The cultures were held above the level of the eye and viewed from beneath at each observation; the white radicles showed up distinctly and it was easy to decide whether a seed had germinated or not. All germinated seeds

were removed as they were found and their number was recorded. The seeds were never touched with the fingers, either in the original sorting, in placing them on the plates, or in removing or transferring them; bone-tipped forceps were always employed. The tests were discontinued before all the seeds that might have germinated had done so, because the 2-hour increments of germination percentage became small and highly variable with longer incubation periods. Satisfactory data on these later increments might have been secured only with a much larger number of repetitions than was needed to give significant values for the earlier ones.

These seeds were only rarely observed to be attacked by molds; even at the highest temperature tested such contamination was slow to appear. Whenever an ungerminated seed was seen to be attacked by mold to such an extent as was judged to threaten the contamination of neighboring seeds it was removed. Sometimes such moldy seeds were discarded but in many instances they were simply transferred to vacant depressions in the same agar plate, left by the previous removal of germinated seeds. Usually these transplanted seeds proceeded to germinate without noticeable retardation.

For this study a seed was considered to have germinated when the radicle had just broken the testa. The radicle usually grew downward within the seed coat and emerged on the lower side.

For recording germination percentages, the experimental unit was usually a set of five agar plates bearing altogether 100 seeds, 20 on each plate. The total number of seeds that had germinated in any set before any observation was consequently the germination percentage for that set and for the corresponding temperature and length of incubation period. Each test comprised four of these 100-seed sets, all incubated simultaneously at the same maintained temperature. Consequently there were four primary percentage values for each period of incubation in each test. Every test was repeated until it appeared that the range of variation of the last four primary percentage values for each length of incubation period was well within the range of variation of all corresponding values previously recorded.

There were finally available a large number of comparable primary percentages for each combination of temperature and time. The arithmetical mean and the standard error of the mean were computed for each combination, and these values appear in table I, separated by the usual $\pm$ sign. In this table the number of primary percentage values on which each mean is based is shown in parentheses below the mean. Combinations that showed no germination are indicated by dashes. The formula used by COLLINS (**4**) was employed in computing the standard error, namely,

$$\sigma_M = \sqrt{\frac{\sigma^2}{N-1}}$$

in which σ_M is the standard deviation of the mean (or the standard error), σ represents the standard deviation of individual values, and N is the number of individual values available,—in this case the number of primary percentage values for the temperature-time in question.

TABLE I

GERMINATION PERCENTAGES OF BLACK EYEBROW SOY BEANS WITH DIFFERENT COMBINATIONS OF TEMPERATURE AND INCUBATION TIME

INCUBATION TIME	24.5°	28.5°	33.0°	36.5°	40.0°
hr.					
12	---	0	3.0 ± 0.4 (12)	1.3 ± 0.4 (12)	---
14	---	3.4 ± 0.2 (8)	16.8 ± 0.6 (20)	13.5 ± 0.9 (20)	---
16	---	17.4 ± 2.2 (16)	39.9 ± 0.5 (24)	36.4 ± 1.2 (24)	---
18	6.5 ± 0.3 (12)	38.0 ± 2.0 (16)	55.6 ± 0.9 (20)	56.5 ± 1.2 (37)	3.8 (2)
20	21.6 ± 2.1 (12)	52.6 ± 1.7 (12)	70.3 ± 1.3 (12)	69.4 ± 0.8 (28)	15.0 ± 0.1 (10)
22	37.4 ± 2.2 (12)	67.8 ± 1.9 (11)	76.8 ± 1.5 (8)	76.3 ± 1.2 (21)	39.3 ± 2.2 (10)
24	51.6 ± 2.2 (12)	77.0 ± 1.6 (7)	80.8 ± 1.4 (8)	79.4 ± 1.2 (13)	58.6 ± 1.6 (10)
26	63.4 ± 0.9 (12)	---	---	84.4 ± 1.6 (12)	70.0 ± 1.6 (8)
28	70.6 (4)	---	---	---	72.4 (4)

As might be expected, there was considerable variation in each series of primary percentage values. The characteristics of the variation usually encountered are shown by the following representative frequency tabulation for the combination of 18 hours and 36.5°. (In the preparation of this tabulation the experimental unit was a single agar plate, instead of five plates, and there were 183 comparable primary values. The largest possible value is of course 20 instead of 100.)

Seeds germinated:	5	6	7	8	9	10	11	12	13	14	15	16	17
Frequency:	0	5	9	8	19	26	34	26	22	21	10	3	0

For this series the average number of germinated seeds is 11.1 and the value for the crude mode is 11.0.

These data are presented graphically by the frequency polygon of figure 1, where the possible values for the number of germinated seeds per plate are plotted on the horizontal axis and the frequency with which each

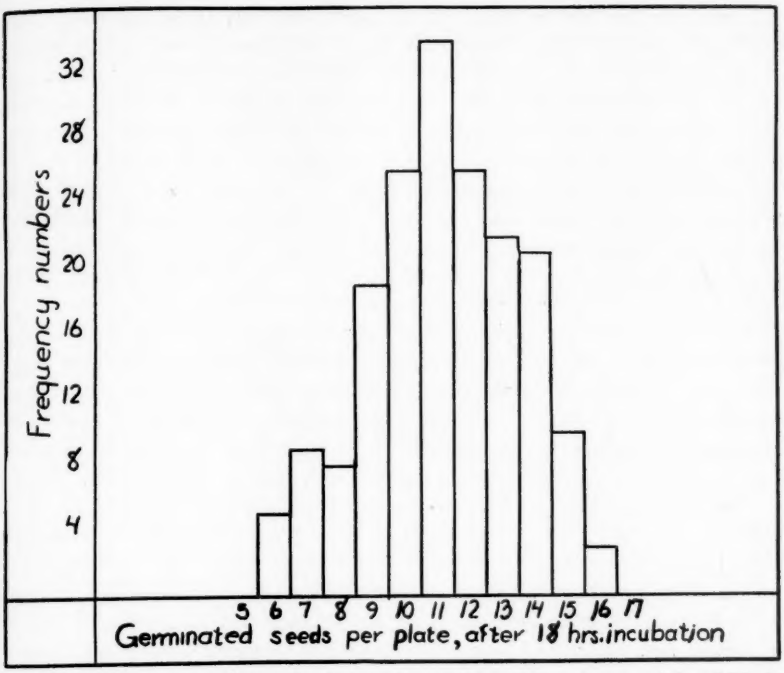

FIG. 1. Frequency polygon showing number of times that various numbers of Black Eyebrow soy bean seeds were found germinated after 18 hours of incubation at 36.5° (based on data from 183 plate cultures, each with 20 seeds).

value occurred is plotted as the height of a rectangle. This polygon indicates that a large proportion of the individual values falls close to the average value (11.1) and in approximately equal numbers on either side of it, and also that any percentage value occurs less frequently as its magnitude is farther removed from that of the average. In these general respects this polygon is similar to the curve representing the theoretical distribution of errors. These observations, and similar ones derived from tests with other temperature-time combinations, appear to justify the use of the averages, each with its standard error, as indices of the general performance of the lot of seeds with which this study deals.

Two sets of criteria might be used for judging the extent of the deviation of the individual values: (1) frequency polygons (such as that of figure 1) constructed from the values used in the computation of the means; and (2) the standard deviation of the means, which are statistical constants. The polygon of figure 1 may serve as a representative sample in detail, but

the tendency for the values of any comparable series to fall close to their arithmetical mean, and hence the relative significance of the latter in representing the whole series, is indicated by the standard error of the mean.

As has been mentioned, each culture was temporarily removed for a short time from its temperature chamber whenever an observation was made; *i.e.*, at 2-hour intervals after the lapse of 10 or 12 hours. All the petri dishes of a set were removed from the chamber together and were returned together after observation. The greenhouse room in which the temperature chambers are, and in which the cultures were examined, had an air temperature usually between 20° and 25° C., and each culture naturally tended to assume the room temperature, becoming either warmer or cooler, while it was out of its chamber. Also, while outside of their chambers the petri dishes and their contents were illuminated, although they were in darkness throughout most of the experimental period. It is conceivable that these short temporary interruptions in the otherwise even tenure of the maintained conditions of temperature and lack of illumination for any culture might exert some sensible influence on germination.

To determine whether this was really so, four special experiments were carried out with a temperature of 36.5°. In each of these experiments there were 25 dishes, 10 of which were removed, examined, and returned to the chamber in the usual way, at the end of 12, 14, 16, and 18 hours while the remaining 15 were not removed and examined until after 18 hours of incubation. For the 18-hour period the average germination percentage was 56.3 ± 1.20 for the first group (which had been subjected to temporary fluctuation) and the corresponding average for the second group was 54.5 ± 1.19. Since the difference between these values cannot be regarded as significant, it may be concluded that the environment changes in question did not alter the course of germination appreciably.

Discussion

General survey of results

Some of the general relations of the average germination percentages for the stock of Black Eyebrow soy bean seed used in the experiments are readily visualized when the averages of table I are plotted as graphs. A simple and direct graphic presentation of these data is shown in figure 2, which embraces seven separate temperature graphs, one for each of the different lengths of incubation period employed. Abscissae are maintained temperatures and ordinates are average germination percentages. Each graph represents the temperature relations of seedling production by this lot of seed in the specified time period. They appear like a group of slightly asymmetrical, downwardly concave curves, with a common maxi-

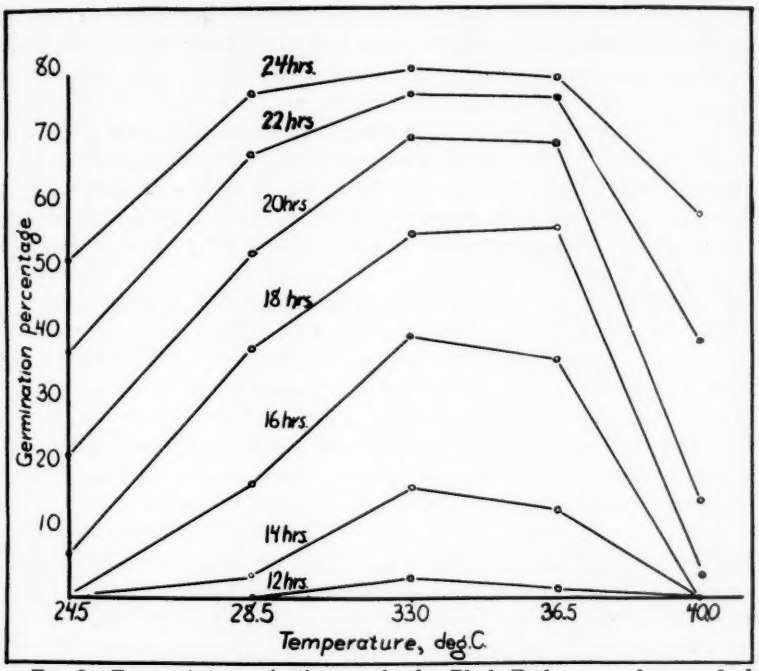

FIG. 2. Temperature-germination graphs for Black Eyebrow soy bean seed, for several lengths of incubation period. Each period length is represented by a separate graph.

mum in the region representing a temperature range from about 33.0° to about 36.5°. Each curve has a steeper slope on the high-temperature side than on the low-temperature side of its peak. For each different length of tested incubation period the temperatures 33.0° and 36.5° were in close agreement with respect to their average germination percentages, but of course the averages are greater as the period was longer. The three other temperatures all gave lower percentages. Cultures at 28.5° gave somewhat lower germination percentages than cultures of the same age at 33.0° or at 36.5°, but those at 28.5° always gave values higher than the corresponding ones at 24.5° or 40.0°. The optimal temperature range for each of the different period lengths consequently extends from about 33.0° to about 36.5°. No shifting of this optimal temperature range with time, such as has been described by several experimenters with other kinds of seed and with various experimental methods, is apparent here.

Successively longer incubation periods broadened the temperature range within which germination was observed. For the 12-hour, 14-hour, and

16-hour periods, minimal and maximal temperatures for the occurrence of germination are apparent, since in these instances two or more of the tested temperatures failed to give any germination. For the longer periods, however, the minimal and maximal temperature values clearly fall outside the temperature range embraced by the tests. With increasing length of period the graphs become progressively flattened at the top and the several tested temperatures tend to approach equal effectiveness with respect to seedling production. WILSON (**40**) found that approximately the same percentage of soy bean seeds eventually germinated at each of the temperatures he tested between 10° and 30°.

In connection with the present study, some experiments with seed of the Manchu, Minsoy, and Tokyo varieties of soy bean showed optimal temperature ranges with upper limits below that of the optimal range for the Black Eyebrow variety as here shown; in most of these additional instances 33.0° was more favorable for germination than either 36.5° or 28.5°.

In addition to the results thus far mentioned, further examination of the data of table I brings out a number of less obvious relations, some of which are considered in the following sections of this paper. It is to be borne in mind that these results and statistical relations apply strictly only to the experiments of this study, to the particular stock of Black Eyebrow seed used, and to the specified conditions and technique of this experimentation. Time factors are of course approximated only in terms of the 2-hour observation intervals, and all critical temperatures, as well as the lower and upper limits of critical temperature ranges, are naturally to be regarded as ranges of temperature rather than as definite temperatures. With more laborious and more time-consuming experimental procedure, and with still further repetitions of the tests, these approximations might have been given a higher degree of precision, of course; but the numerical results appear to be sufficiently precise to furnish some additions to our knowledge of the performance of samples of germinating seeds. The following discussion will be in terms of temperature ranges rather than in terms of exact temperatures, and each critical temperature mentioned may be considered as approximate to within a few degrees. The need for brevity of statement and for general clearness frequently limits the extent to which considerations concerning relative degrees of precision may be mentioned throughout reports on many biological studies. It is interesting to note that the inevitably approximate nature of critical temperatures for seed germination was clearly emphasized by HABERLANDT (**13**) as early as 1874, who wrote:

"Denn die Temperaturgrenzen bei welchen eine Schwächung und Verlangsamung des Keimungsprozesses eintritt, ist keiner scharf gezogene

Linie, sondern einem Streifen zu vergleichen, der unter Umständen eine Verschiebung der Grenzlinie für den Samen einer bestimmten Qualität wohl gestattet. Die mehr oder weniger vollkommene Reife und Ausbildung des Kornes, sein Alter, seine Heimath u. s. w. werden darauf Einfluss nehmen können, daher man mit der Angabe sich wird zufrieden stellen müssen, dass für irgend eine beliebige Samenart die obere Grenze zwischen diesen und jenen Graden schwankte und entweder näher der einen oder der anderen Zahl liege."

Course of germination at maintained temperatures

Although it is interesting to note that the graphs of figure 2, which represent the performance of a population of seeds subdivided into cultures that were incubated at different temperatures, bear a superficial resemblance to many temperature graphs of physiological processes, such as growth, yet it must be remembered that the germination percentages of this study do not refer to process rates in individual seeds. The percentages are statistical values representing the various proportions of a seed population that were able to attain a specified developmental stage in cultures of certain ages at the specified temperatures. These proportions are dependent, not only upon prevailing environmental conditions and the duration of incubation, but also upon differences in physiological behavior among the individual seeds constituting the population. The percentage values provide a means for analyzing the physiological variability of the individual seeds within the population and they will now be considered with reference to several types of analysis designed to reveal some features of the nature and extent of this diversity.

In the graphs of figure 3, germination percentages are represented as ordinates and lengths of incubation time are abscissae, the data for each temperature being plotted separately. The form of each graph shows how the original seed population became gradually transformed, with lapse of time, into a population of seedlings. For all five tested temperatures this transformation evidently followed the same general course. All five graphs are sigmoid and their respective apparent points of inflection are indicated by circles. In general, the cumulative germination percentage increased slowly during the earlier observation periods, and then more rapidly, until a percentage corresponding to the apparent point of inflection of the graph was reached, after which the rate of increase fell off more and more. The five graphs are so similar that if they were plotted as arising from a common abscissa they would nearly coincide throughout. One feature of their close similarity is well shown by the fact that the percentage values for their apparent points of inflection all fall within the narrow range from about 36.4 to about 39.9.

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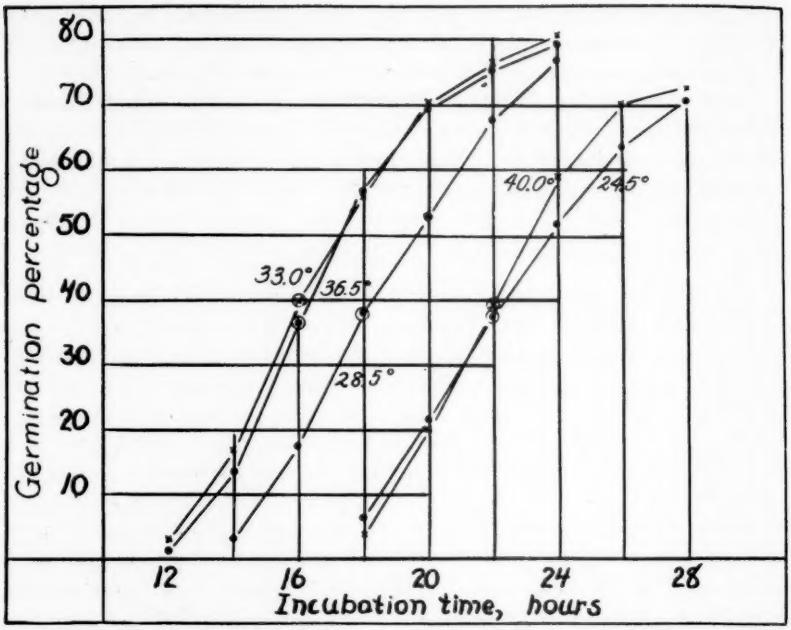

FIG. 3. Graphs for Black Eyebrow soy bean seed showing germination-percentage values as related to incubation time. Each graph represents a different temperature. Approximate points of inflection shown by circles.

The principal differences among these graphs are with respect to the time periods they represent. The graphs for 33.0° and 36.5° are seen to be almost identical in this regard and those for 24.5° and 40.0° are not greatly divergent, but the graph for 28.5° stands by itself. Less than 12 hours of incubation at 33.0° or 36.5° sufficed for the appearance of the first seedlings; in cultures at 28.5° the first seedlings were observed only after 14 hours of incubation; and seedlings did not begin to appear in cultures at 24.5° or 40.0° until these had been incubated for 18 hours. This same relationship between incubation time and temperature persisted throughout this study; any given percentage value was attained in cultures at 33.0° or 36.5° about two hours earlier than in cultures at 28.5°, and about six hours earlier than in cultures at 24.5° or 40.0°.

This kind of graph, showing progress of seed germination as related to lapse of time, has not been employed by many writers in this general field; but many papers give tabular data from which graphs of this sort may be constructed, and such graphs would generally be similar in their essentials to those shown in figure 3. Among the publications in which special

attention is given to this kind of analysis may be mentioned HARRINGTON'S (**16**, p. 348) germination-time graphs for his lot of seed of *Impatiens balsamina*, which present important relations and do not differ in general form from those of figure 3. The graphs given by EVANS (**7**), derived from seed of *Amaranthus retroflexus* germinated at nine different temperatures, show more variability in form than those of figure 3. A temperature of 42° gave the highest time rate of seedling production. The first seedlings appeared later in cultures at 46.1° than in those at 42°, and the cumulative increase in germination percentage was slower in cultures at 46.1°.

INCUBATION TIMES CORRESPONDING TO REPRESENTATIVE PERCENTAGE VALUES

As an alternative to representing the data of table I in terms of germination percentages attained after incubation periods of definite length, it is possible by interpolation to obtain from these data the lengths of time that correspond to certain definite germination percentages. The results of such a series of interpolations are shown by the graphs in figure 4, in which lengths of incubation time are measured on the vertical axis while temperature is indicated on the horizontal axis. There are three graphs, for 3, 38, and 70 per cent. respectively. These particular values are selected for special study because mean percentages of about these magnitudes appear for several temperatures in the table, and because the percentage value 38 is especially interesting since it represents the proportion of seeds that had germinated before the rate of seedling production began to fall off (see figure 3, the points of inflection). The graphs of figure 4 are concave upward, the low-temperature ends of the ones for 3 and for 70 per cent. being farther apart than the high-temperature ends. This difference between the lengths of time corresponding to these two percentages decreases as higher temperatures are examined, and it is interesting to note that there is no evident alteration of this relation as the optimal range of temperatures is approached and passed. These graphs suggest that an optimal range of temperature for seed germination might be taken as the range for which the least time is required for the germination of a specified proportion of the lot of seed studied. Thus the temperature range 33.0°–36.5° might be considered as optimal for the percentage range 3–70, according to the graphs of figure 4.

These graphs may be viewed as contour lines on a 3-dimensional diagram showing the relations of temperature, incubation time, and germination percentage. For each germination percentage many different lengths of incubation period are each seen to have two separate temperature ranges equally favorable for seedling production, one of these being suboptimal and the other supraoptimal.

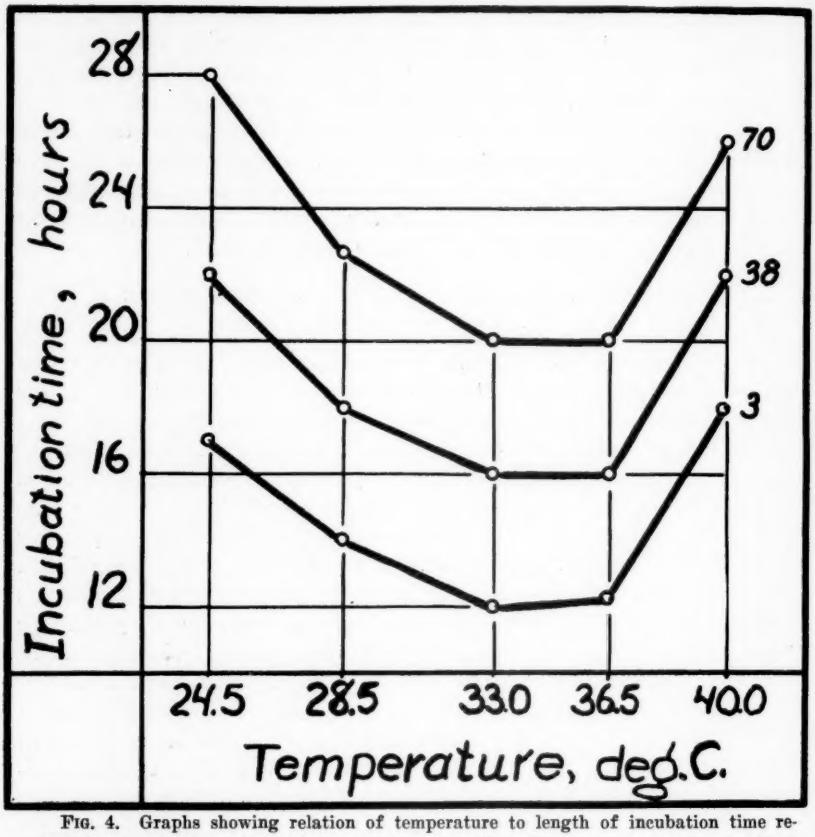

FIG. 4. Graphs showing relation of temperature to length of incubation time required for germination of specified percentages of Black Eyebrow soy bean seed. Each graph represents a different germination percentage.

Similar relations of incubation time to temperature have been reported by several workers. In the course of experiments on temperature relations of nuclear phenomena, HARTMANN (**17**) made some preliminary germination tests at eight constant temperatures between 8.5° and 41.0°, using moist sawdust as substratum. For seed of *Phaseolus multiflorus, Helianthus annuus,* maize, and pea, 31° induced the earliest appearance of seedlings; at higher and lower temperatures seedlings appeared later. BREMER's (**3**) results for germinating beet seed show an optimal temperature range (22°–36°) for which the production of seedlings required only one or two days of incubation; with higher and lower temperatures longer

incubation was necessary. REYNOLDS (**32**), KOTOWSKI (**21**), WILSON and HOTTES (**41**), and WILSON (**40**) noted a diminution in the incubation period necessary for germination as higher temperatures were examined, but these tests, which embraced no temperatures above 30°–32°, did not involve temperatures high enough to induce retardation of germination. HABERLANDT (**13, 14**), KREYSING (**22**), and BÄR (**2**) also found definite evidence of a retardation in seedling production at high temperatures as compared with lower ones. LIVINGSTON and HAASIS's (**24**) graphs showing the relations of temperature, incubation period, and germination percentage for their French rice seed are similar to the graphs of figure 4, but the range of period length between the beginning of germination and the attainment of 90 per cent. is shown to be narrower for the optimal temperature (about 34°) than for suboptimal or supraoptimal temperatures.

INCREMENTS OF GERMINATION PERCENTAGE

It is evident that the seeds tested in this study varied considerably in rate of response to uniform environmental conditions, because if all the seeds had possessed identical physiological properties every radicle should have emerged after the same period of incubation at any given temperature. Although indications as to the nature of the diversity existing within a seed population may be obtained by inspection of sets of graphs such as those of figure 3, some features of this diversity may be more readily appreciated if the increments of germination percentage from observation to observation are employed. Such percentage increments, computed from the data of table I, are set forth by means of the histograms of figure 5, in which the 2-hour observation intervals of the incubation period are indicated on the horizontal axis while the increments in question are plotted vertically, each of their values being inscribed just above its horizontal segment of the graph. This set of graphs thus constitutes a table of values arranged in graphic form. Each of the five temperatures is represented by a separate graph.

Increments of germination percentage may be treated in either of two ways. (1) Attention may be focused on the fluctuating rate at which the original seed population is transformed into a seedling population and the average rates for the several 2-hour intervals may be compared to bring out acceleration and retardation. Since counts of germinated seeds were made at each observation, these rates for the several intervals are to be read directly (as percentages of the seed population) from the graphs of figure 5. Or (2) the percentage increments may be considered as measures of one kind of physiological diversity which was latent in the seed population. The histograms of figure 5 thus become frequency polygons that show

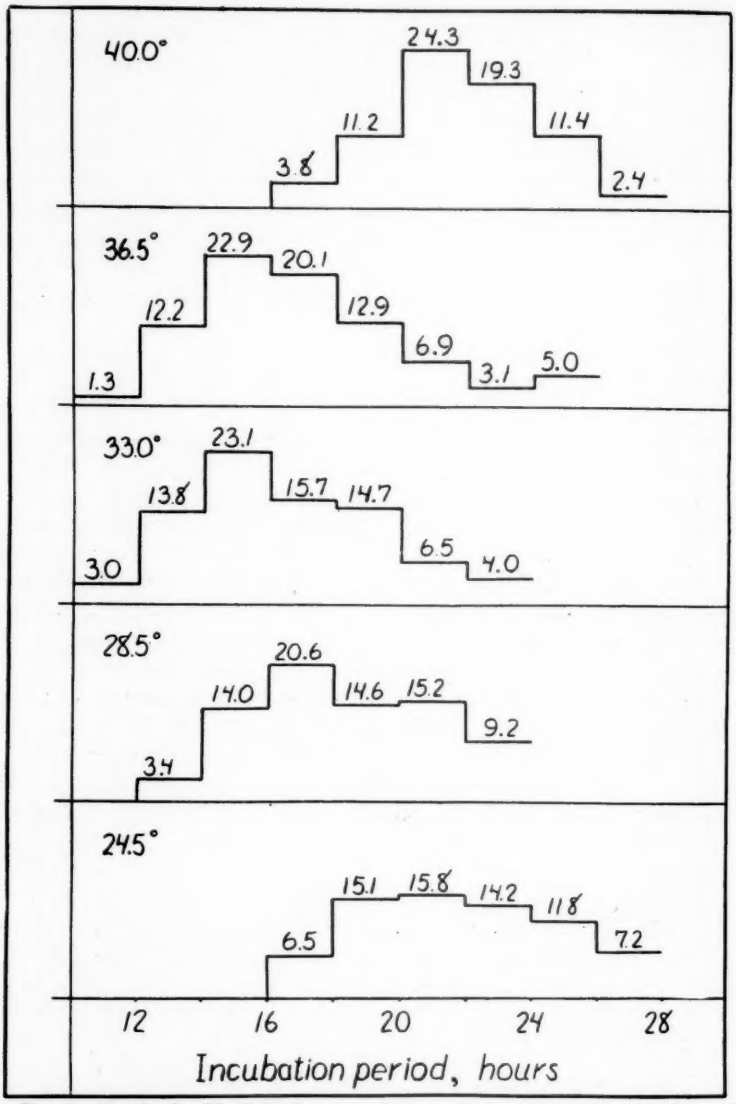

FIG. 5. Graphs for Black Eyebrow soy bean seed showing increments of germination percentage for 2-hour intervals. Each graph represents a different maintained temperature.

the relative proportions of the population that were embraced in several physiological classes of seeds. Since both these modes of treatment are instructive, the 2-hourly increments of germination percentage will be discussed in terms of each, in turn.

RATES OF SEEDLING PRODUCTION.—The rate of seedling production is seen (fig. 5) to have changed in a rather regular way for each of the five temperatures tested. Beginning with a low value for the first interval that yielded any seedlings at all, it increased rapidly to a maximum, shown for the third 2-hour interval of seedling production, and then it decreased. For the three lower temperatures the rate for the fourth interval is about like that for the second, and for the two higher temperatures this is true of the rate for the fifth interval. For subsequent intervals the rate generally continued to decrease progressively. The decrease is usually seen to have been less rapid than the earlier increase; *i.e.*, retardation of the rate of seedling production was less rapid than acceleration. The differences between successive values of the rate are more marked for the higher temperatures than for the lower ones; for 24.5° there is relatively little difference between the 18th hour of incubation and the 26th hour, and no doubt this tendency toward uniformity of rate throughout many consecutive 2-hour intervals would be still more pronounced if still lower temperatures had been employed. There was evidently a direct relation between the incubation temperature and the maximum rate of seedling production, the rate for the third interval after seedlings began to appear being greater as the temperature was higher.

Since the peaks of the graphs of figure 5 mark the respective points at which seedling production began to fall off, the abscissa of the peak for each temperature is equivalent to the abscissa of the apparent point of inflection on the corresponding graph of figure 3. Both figures consequently show the same relations between incubation time and the maximal rate of seedling production for each tested temperature, for the rate of seedling production is of course just the rate of increase in the cumulative germination percentage in each series. Table II shows these relations.

Although the maximal 2-hour rate of seedling production and the duration of incubation required for the development of that maximal rate are both seen to have varied considerably and consistently with the degree of temperature employed, yet the total germination percentage corresponding to the apparent points of inflection on the graphs of figure 3 (and hence to the maximal rates of seedling production) appears to have shown no significant and consistent variation with temperature. This apparently critical percentage value is constant within the narrow range from 36.4 to 39.9, and its average magnitude for all five temperatures is 38.2. This is a remarkable constancy, and it is at once suggested that we have here a critical

TABLE II

MAXIMAL 2-HOUR RATES OF SEEDLING PRODUCTION AND CORRESPONDING GERMINATION PERCENTAGES AND LENGTHS OF INCUBATION PERIOD AT DIFFERENT TEMPERATURES DERIVED FROM FIGURES 3 AND 5

MAINTAINED TEMPERATURE	MAXIMAL 2-HOUR RATE OF SEEDLING PRODUCTION (FROM FIG. 5)	GERMINATION PERCENTAGE CORRESPONDING TO MAXIMAL RATE OF INCREASE IN GERMINATION PERCENTAGE (FROM FIG. 3)	INCUBATION PERIOD
°C.	%	%	hr.
24.5	15.8	37.4 ± 2.2	22
28.5	20.6	38.0 ± 2.0	18
33.0	23.1	39.9 ± 0.5	16
36.5	22.9	36.4 ± 1.2	16
40.0	24.3	39.3 ± 2.2	22

index of some characteristic or combination of characteristics of this lot of seeds. Apparently about 35–40 per cent. of the individuals were able to germinate with exceptional promptness, no matter which of the five widely different temperatures was employed. Whether the group of specially prompt and highly temperature-tolerant seeds (with respect to their capacity for germination) would embrace the same individuals for all favorable test temperatures is of course not known, for this apparently significant percentage is merely a statistical value pertaining to the entire lot.

Such an index may perhaps be of value in practical seed testing, for useful viability indices of stocks of seed may well refer definitely to capacity for prompt (although incomplete) germination of representative samples under a variety of environmental conditions, rather than to capacity for ultimate (and more nearly complete) germination of the samples with an environmental complex that is specially favorable to germination without reference to promptness. Several investigators mentioned by TOUMEY (**37**, p. 128) have recognized that the percentage of seeds germinating before the rate of seedling production begins to fall off may be used to represent the capacity of a given lot of seeds to produce seedlings quickly. In many situations encountered in forestry and agriculture, plant competition is so severe that an early start may be essential if a plant is to survive. TOUMEY used the name "germinative energy" for this percentage value, a term not wholly satisfactory, since ambiguity in the broader usages of science is apt to arise when a word (such as energy) with a definite meaning in one field (physics) is used in a different sense in another field (biology).

PHYSIOLOGICAL CLASSES OF SEEDS.—If attention be directed to the time spans covered by the process of extrusion of radicles, the degree of diver-

sity of individual seeds with respect to rate of response to the prevailing germination conditions becomes more apparent. For instance, the first seedlings were found after 12 hours of incubation at 33.0°, and after 24 hours only 80 per cent. of the population had germinated. It seems evident that there must have been important physiological differences of some kind among the seeds of this lot, when, for example, some seeds required only 12 hours and others required 24 hours to reach the same stage of development, although all were treated alike.

Since the cultures were examined every two hours and all germinated seeds were removed at each examination, this procedure operated as a sorting process, the seeds falling into one or another of several different physiological classes. Each class was characterized by rates of response of its members to specified conditions of incubation. If the histograms of figure 5 are examined with this concept in mind, then the intervals on the horizontal axis represent the various physiological classes into which the seeds were sorted, and the height of each segment or block of the histogram indicates the relative size of its particular class. Comparison of the five histograms brings out some notable similarities. Each shows a tendency for the values for length of incubation time to fall close to a crude mode (the third interval measured from the beginning of the appearance of seedlings), and the farther removed a class is from the crude mode the fewer are the individuals in it. Furthermore, the number of individuals with longer incubation periods than the modal length exceeds the number with shorter incubation periods, and each graph is consequently somewhat asymmetrical. In these respects the histograms are not unlike many other frequency polygons representing the distribution of some quantitative character among the members of a population of organisms. In connection with the study here reported, similar data were obtained for several additional kinds of seed, including three soy bean varieties and *Alisma plantago-aquatica* L.; in nearly all instances where large seed samples were employed with a maintained temperature, a frequency polygon essentially much like those shown in figure 5 could be constructed from the experimental results. Perhaps the most remarkable feature of these histograms representing a grouping of the seeds into physiological classes is their evident similarity with respect to the size relations of the several classes. That this sort of graph takes so nearly the same form for all five temperatures renders this relation much more striking and apparently more significant than it might be if all tests had been carried out at the same temperature.

It will be observed that the higher-temperature graphs of figure 5 exhibit more abrupt steps or gradations from class to class than are shown by the lower-temperature graphs. This is probably related to the fact that the processes of germination went forward more rapidly at higher tempera-

tures than at lower ones. Obviously the results of this kind of sorting should depend not only on the statistical distribution of the different kinds of individuals in the original seed sample, but also on the time-temperature characteristics chosen for the sorting procedure. For example, if it were required to sort 80 per cent. of the seeds of a sample into a specified number of classes, and if the relative sizes of the several classes were also specified, then this sorting technique might be applied by using any one of a number of different maintained temperatures, but the observation intervals would need to be shorter for higher temperatures than for lower ones.

Probably the properties of the agar gel on which the seeds rested changed during incubation, and the slowly developing seeds may not have been subjected to exactly the same environmental conditions as the rapidly developing ones. If it had been possible to maintain the environmental conditions constant throughout the period of incubation, perhaps the rates of seedling production might have been somewhat different. If greater changes in the medium took place at high temperatures than at low ones, some of the differences in form of the graphs of figure 5 might be accounted for. Such considerations as these, however, cannot alter the significance of the fact that the rates of response of the individual seeds were not alike, for all the seeds of any culture received similar treatment at the start.

Although many students of seed germination have been aware that counts made at intervals in a germination experiment furnish data resolvable into graphs similar to those of figure 5, nevertheless this aspect of the general problem of germination relations has rarely been discussed. Perhaps one reason for this may be that many experimental studies on seed germination have been made with relatively small seed samples. With small samples the increments of germination percentage are naturally also small, and they are likely to be irregular as well. In the present study the seed samples were relatively large (from 800 to 3600 individual seeds per sample), and this seems to have insured for every sample a fair representation of all the physiological classes in the stock, smoothing out many irregularities of the component tests,—irregularities that might have obscured important relations if the samples had been much smaller.

EARLIER STUDIES ON PHYSIOLOGICAL CLASSES OF SEEDS IN SAME STOCK.—GOODSPEED (**11**) made daily germination counts on lots of seed from a great number of plants in his breeding experiments on tobacco, and showed how the daily percentage-increment values altered throughout a long experiment period. Recognizing that the regular removal of germinated seeds from the cultures results in a physiological classification, GOODSPEED proposed this as a method for analyzing the genetic constitution of the progeny of hybrid plants. His results refer to seed samples that were not very large (100–200 seeds per sample), and some of the irregularity in the relative magnitudes

of his increments may have been due to inadequate sampling. Expressed in the form of frequency polygons, his data take a variety of forms; most of the graphs show only one mode, but those for some samples of seeds from hybrid parents show two modes. A few are symmetrical and there is no apparent agreement among the others with regard to relative steepness on the two sides of the mode.

Although not concerned with seed germination, LOEB and NORTHROP'S (**25**) data on the temperature relations of the development of fruit flies (*Drosophila*) are of interest here. Flies were grown from eggs under controlled conditions at several constant temperatures between 10° and 31.5°. At regular intervals each culture was examined and record was made of the number of larvae present. Similar observations were subsequently made on pupae and on the emergence of imagoes. For each stage of development the same kind of individual variation was noted; the rate of appearance of individuals at any given developmental stage rose to a maximum and then fell off, usually more slowly than it had risen. Figure 6 shows the temperature-time relations of the emergence of *Drosophila* imagoes, the daily emergence-percentage increments for which have been computed from LOEB and NORTHROP'S tables. These histograms are plotted like those of figure 5 and require no special explanation. Rates of emergence are an expression of individual variation in capacity for development, and a study of this kind shows not only the constitution of the population under consideration but also the extent to which the units of the population are influenced by the various sets of conditions employed in the experimentation.

WAGGONER (**39**) studied the progress of germination in samples of radish seeds with various water contents, the samples having been subjected to a temperature of 80° for 30 minutes before being distributed to germinate on wet plaster-of-Paris blocks. Samples with original water content of 18 per cent. failed to show germination, and those with 14 or with 9 per cent. moisture showed limitation and retardation of germination as compared with the sample having water content of only 4 per cent. Daily counts of germinated seeds showed clearly the manner in which the time rate of seedling production increased to a maximum and then decreased. Histograms constructed from WAGGONER'S tables possess the same general characteristics as those shown in figure 5.

This germination method of separating physiological classes in a stock of seed has been used to advantage by CORRENS (**5**). Seeds from artificially pollinated plants of *Melandrium* (a dioecious form) were incubated on moist paper and the seedlings transplanted to soil as they appeared, being classified into quartiles on the basis of the time required for their emergence from the seed. Growing 11,558 of these seedlings to a developmental stage

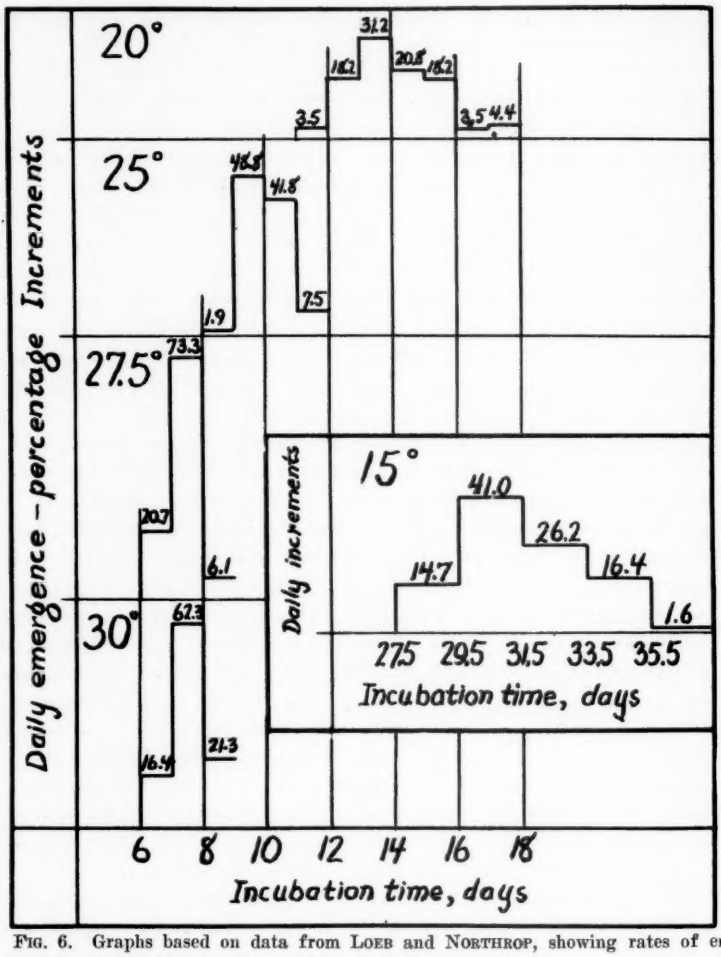

FIG. 6. Graphs based on data from LOEB and NORTHROP, showing rates of emergence of *Drosophila* imagoes at several maintained temperatures.

that permitted the staminate and pistillate individuals to be separately counted, CORRENS found that seeds destined to produce staminate plants tended to germinate more promptly than the others. In the progeny of one staminate parent the percentage of pistillate individuals in the first quartile (most rapid germination) was 32.8; in the second, 52.5; in the third, 62.6; and in the fourth (representing the seeds that had germinated most slowly), 69.0. Incidentally he observed that the rate of emergence

of seedlings rose to a maximum on the second or third day of incubation and declined thereafter.

PIEPER (**30**) observed that there was no correlation between the rate of germination of parents and of progeny in several samples of *Poa pratensis* seed from which he raised plants and obtained seed. He noted, however, that seeds from slowly germinating parents tended to germinate slowly, and that plants from rapidly germinating seeds produced seeds that tended to germinate rapidly.

PEARL (**28a**) observed differences in growth rate among seedlings sorted into physiological classes by methods similar to those used in the present study. After preliminary soaking, *Cucumis melo* seeds were sown on agar plates, incubated in darkness at 30°, and observed several times during the period of emergence of radicles. At each observation all germinated seeds were transferred to fresh agar plates and allowed to grow for an additional 48 hours. At the end of this growth period each group of seedlings was divided into cotyledons, hypocotyl, and roots, measurements of moist and fresh weight being made. The combined weight of hypocotyl and roots was taken as a measurement of growth. He found that the seeds which took the longest time in germinating made the least growth subsequently, and the best growth was made by the classes of seeds that germinated rapidly.

Both HAASIS (**12**) and TANG (**36**) called attention briefly to the possibilities of this sort of physiological technique for the sorting of a sample of seed into classes, but they gave only very limited attention to the question concerning the physiological classes of seedlings. The possibility of employing the temperature-time relations of seed germination as criteria in selecting similar lots of seedlings for physiological experimentation has been emphasized by LIVINGSTON and HAASIS (**24**).

Variation in the physiological characteristics of seeds may be manifested in other ways besides considering rates of seedling production. PORODKO (**31**) contributed information concerning the death rates at high temperatures, and SIFTON (**34**) reported on the death rates of seeds during storage. For each of these sets of data the decrement of the germination percentage may be studied and graphs similar to those of figure 5 may be constructed which bring out interesting relations. If, like HEWLETT (**18**), one suitably applies a toxic substance to a seed population for a given time period some of the seeds die, and these constitute a class whose individuals must have been characterized by about the same degree of resistance; if a suitably longer exposure period is used the seeds of still another class are killed, and so on. Naturally, if seeds must be killed in order to study their physiological variation, this method may be regarded as of limited usefulness in some respects; but the results secured by its application provide additional means

for comparing different lots of seed with respect to their intrinsic physiological variability.

Mean length of incubation time

Haberlandt (**14**), Pieper (**29**), Akemine (**1**), Gassner (**9**), and Kotowski (**21**) expressed the time relations of the germination of their seed samples by means of a mathematical expression or index that has considerable promise of usefulness when different lots of seed are to be compared. This expression is the mean length of incubation time required by the seed sample in question, for germination under specified test conditions. Kotowski used the reciprocal of the mean length of incubation time, calling it the "coefficient of velocity" of germination.

To derive the mean length of incubation time, a seed sample is tested in the manner employed in the present study, with frequent observations and records of the germination-percentage increments for the several observation intervals. Then each percentage increment is multiplied by the corresponding length of incubation period, measured from the beginning of the test, and all resulting products are summed. Finally, the sum thus secured is divided by the total germination percentage for the whole test; that is, by the sum of all percentage increments. For example, consider the percentage increments and the corresponding incubation times for the seeds of the present study, and for the maintained temperature 24.5° (fig. 5). The six increment-time products are $6.5 \times 18 = 117.0$, $15.1 \times 20 = 302$, $15.8 \times 22 = 347.6$, $14.2 \times 24 = 340.8$, $11.8 \times 26 = 306.8$, and $7.2 \times 28 = 201.6$. The sum of these products (1615.8) is divided by 70.6 (the sum of all six increments, or the final germination percentage) and the resulting quotient is 22.9 hours, which is the required mean incubation time for all tests at 24.5° carried out in this study. These individuals may therefore be said to have required, on the average, an incubation period of 22.9 hours for germination at 24.5° and under the background conditions of the experimental technique employed in these tests.

This mean has different values according to the five different maintained temperatures employed: for 24.5° it is 22.9 hours; for 28.5°, 19.4 hours; for 33.0°, 17.5 hours; for 36.5°, 18.1 hours; and for 40.0°, 22.8 hours. These values are slightly greater than the corresponding incubation times represented by the peaks of the graphs of figure 5, and when plotted their graph is very similar in form to the graphs of figure 4.

Because this index of germination varies with the maintained temperature used in the experiment from which it is derived, it may furnish an additional criterion for establishing a temperature optimum for the germination of a stock of seed, that temperature being taken as optimal for which the lowest mean incubation time is shown. Thus from the values

just given, it appears again that a temperature optimum may be considered as between 33.0° and 36.5°; the difference between the two index values 17.5 (33.0°) and 18.1 (36.5°) probably being insignificant.

When several stocks of seed are to be compared as to average promptness of germination, they might be tested severally in a manner similar to that of this study and a score number or coefficient of germination capacity for each stock might be the mean incubation time corresponding to the optimal temperature for that stock; *i.e.*, the shortest mean incubation times shown by the several stocks, or the reciprocals of these time values, might be useful indices for the comparison of different stocks. Many other uses for this mean incubation time might be suggested.

Summary

1. Samples of a stock of seed of Black Eyebrow soy bean were incubated on 0.5-per cent. agar plates at five maintained temperatures (24.5°, 28.5°, 33.0°, 36.5°, and 40.0°) and the number of germinated seeds recorded at 2-hour intervals, until about 80 per cent. of all the seeds of a test had germinated. According to several different criteria, the optimal temperature range for germination was found to be 33.0°–36.5°. Within this range (a) the least time was required for the appearance of any given germination percentage, (b) the greatest percentage of seeds germinated during any of the incubation periods tested, and (c) the mean incubation time was shortest.

2. The rate of seedling production, measured as the percentage of the total seed population germinating per 2-hour interval, showed a maximum for the third 2-hour interval in which any seedlings were found. For each of the five temperatures tested the germination percentage attained when the rate of seedling production began to fall off was found to be 35–40. For later incubation intervals the rate of seedling production decreased more slowly than it had increased prior to the attainment of its maximal value.

3. The removal of germinated seeds from test cultures at regular intervals constitutes a sorting process which distributes the seedlings derived from a seed sample into several classes, according to their rates of emergence under specified uniform and favorable sets or complexes of germination conditions. In terms of this concept, the increments of germination percentage from one observation to the next indicate the relative sizes of these physiological classes in the original seed sample as well as in the resulting lot of seedlings.

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FACTORS AFFECTING ASSIMILATION OF AMMONIUM AND NITRATE NITROGEN, PARTICULARLY IN TOMATO AND APPLE

VICTOR A. TIEDJENS[1]

(WITH SIX FIGURES)

Introduction

Plants absorb ammonium and nitrate nitrogen in varying quantities, depending partly on the rate of assimilation[2] of either ion in the plant. That certain specific conditions are necessary for the most efficient assimilation of either form of nitrogen has been pointed out (**40**). Different species vary in the efficiency with which they utilize the nitrate or ammonium ion under a given set of conditions (**29**). The hypothesis that both ions probably are finally utilized as ammonium, even though external requirements may be different, involves an interesting study, a preliminary report of which has been published (**40**). A continuation of these studies has established certain relationships affecting the assimilation of nitrate and ammonium nitrogen. Such factors as hydrogen-ion concentration and available carbohydrates have a direct bearing on the assimilatory processes. That these relationships vary with the source of nitrogen, and that the location of the initial assimilatory processes varies with the type of plant, will be pointed out.

Experimental methods

Tomato and cotton plants, and small 1-year-old Delicious, Stayman, and Baldwin apple trees, all root-grafted, were grown in sand cultures. The nutrient solutions were modifications of one of the ammonium sulphate series recommended by JONES and SHIVE (**12**), the compositions of which are shown in table I.

Calcium and sodium salts were used as the source of nitrate, whereas ammonium sulphate, ammonium carbonate, and ammonium hydroxide were used as the sources of ammonium. Details of growing the plants for some of the experiments have been discussed elsewhere (**39, 40**).

Experiments were conducted with the tomato (*Lycopersicon esculentum* Mill.) cotton, and apple (*Pyrus malus* L.) in which the pH of the solutions percolating through the sand was maintained within 0.2 of the initial pH.

[1] The writer acknowledges his indebtedness to G. T. NIGHTINGALE, SANTE MATTSON, and J. W. SHIVE for their helpful suggestions during the progress of the experiments and the preparation of the manuscript.

[2] Assimilation in this report refers to the reduction and elaboration of the nitrate and ammonium ions after they have been absorbed by the plant.

TABLE I

PARTIAL VOLUME MOLECULAR CONCENTRATION OF COMPOUNDS IN NUTRIENT SOLUTIONS. INITIAL pH OF NUTRIENT SOLUTIONS WAS MODIFIED TO SATISFY REQUIREMENTS OF EXPERIMENTS WITH 0.1N SULPHURIC ACID OR POTASSIUM HYDROXIDE UNLESS OTHERWISE STATED

SERIES	pH*	KH_2PO_4	$CaCl_2$	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	$NaNO_3$	$MgSO_4 \cdot 7\ H_2O$
A	3–9 incl.	0.00633	0.00292	.....	0.00584	.....	0.00237
B	3–9 incl.	0.00633	0.00292	.....	.....	0.01168	0.00237
C	4–9 incl.	0.00633	0.00292	0.00560	.....	.....	0.00237
D*	6.4–9 incl.	0.00633	0.00292	.....	.....	.....	0.00237
E	8.0	0.00530	0.00146	0.00840	.....	.....	0.00118
F	8.0	0.00633	0.00584	0.00140	.....	.....	0.00237
G	5.1	0.00633	0.00584	.....	0.00146	.....	0.00237

* Number of cc. of $\frac{M}{2}$ ammonium hydroxide per liter for each pH as indicated: pH 6.4 = 1, 6.8 = 2, 7.3 = 3, 7.8 = 4, 8.4 = 6, and 8.8 = 8 cc. The pH 8.4 cultures did not receive quite so much nitrogen as the C cultures supplied with ammonium sulphate.

In these cultures from 10 to 24 liters of nutrient solution, varying with the size of the plants, were supplied to the sand cultures every 24 hours. This was accomplished by running 5 to 12 liters of solution through in 12 hours. The solution after dripping from the cultures was adjusted to the desired pH and used several times. The solutions were changed every third day. Solution lost by evaporation and transpiration was replaced by fresh nutrient solution. The osmotic concentrations varied slightly during the 3-day period that the solutions were used. Because of the large amount of nutrient solution required, the experiments necessarily were limited in scope, but were sufficiently comprehensive to establish the constant pH at which the plants would grow with nitrate and ammonium nitrogen, and supplemented those experiments from which the data have been published (**39, 40**).

EXTRACTION OF PLANT TISSUES BY CHEMICAL METHODS.—Two methods were employed in obtaining an extract of ionizable substances from the plant tissue, namely, aqueous extraction of the fresh tissue, and extraction of the fresh tissue by electrodialysis, which separated the anions from the cations into two separate solutions. When time did not permit immediate extraction of the fresh tissue, it was weighed into quart Mason fruit jars, sealed, and stored at -6 to $-20°$ C. until analyzed. The freezing method employed has been found to be satisfactory for this purpose (**24**). Handling the tissue in this manner made it possible to harvest an entire series of twenty or more samples and analyze them as time permitted.

EXTRACTION OF PLANT TISSUE BY ELECTRODIALYSIS.—The three-compartment electrodialyzer as employed and described by MATTSON (**16**) was found satisfactory for obtaining extracts of plant tissue. Cations were removed by a copper cathode and anions were removed by means of a platinum anode. Most of the amino acids, amides, and polypeptide groups were collected in the cathode chamber. An advantage of this method of extraction was the separation of nitrate nitrogen from the other nitrogenous fractions, thus eliminating nitric acid, which apparently interferes with the analysis of other nitrogenous fractions (**39**). Electrodialysis also left the more complex proteins in the central chamber. The solution containing the cations was usually clear, but in some cases contained a yellow pigment. The solution containing the anions was clear and usually colorless. Ammonium and other monovalent cations were extracted from the tissue in a very short time, whereas the anions were removed much more slowly. This has been shown by MOORE, REEVES, and HIXON (**19**). In the present studies it was found that ammonium ions were almost completely removed in 15 minutes.

The protein content in the tissue remaining in the central chamber was usually slightly lower in the electrodialyzed sample, and the soluble organic

and inorganic nitrogen was higher than in the residue of sample extracted with water and coagulated with dilute acetic acid. This was accounted for by difference in the efficiency of the two methods in extracting nitrates and ammonium ions, which will be shown by data to be published later. Amide nitrogen was entirely removed only after carefully spacing the electrodes and removing the dialysate from the cathode chamber at frequent intervals. Amides and amino acids will take on a positive or negative charge, depending on the pH of the solution in which they are dissolved, and will then tend to move to the pole of opposite charge. Asparagine has

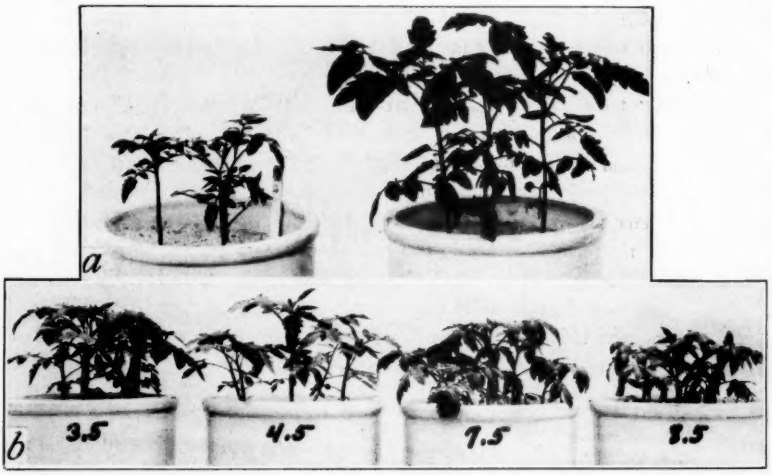

FIG. 1. *a*, tomato plants of experiment I in sand cultures, initially extremely high in carbohydrates, after 3 weeks with a complete nutrient solution containing sodium nitrate at initial pH 5.4 (left) and ammonium sulphate at initial pH 8.0 (right). *b*, tomato plants grown with sodium nitrate at initial pH 3.5 and 4.5 (left) and ammonium sulphate at initial pH 7.5 and 8.5 (right). These plants received the nutrient solution as soon as the first true leaf appeared.

an isoelectric point of pH 4.0 to 4.2. Asparagine in tomato sap at pH 5.4 probably has a negative charge; but when placed in the electrodialyzer, because of the rapid extraction of the monovalent cations, the material containing the asparagine in the central cell becomes very acid, causing the asparagine to take on a positive charge. It then tends to move in the direction of the cathode chamber, which by this time is definitely alkaline (above pH 8.0). Unless the solution from the cathode chamber is immediately removed, the charge on the asparagine is reversed and it will travel back to the central chamber. Amino acids with higher isoelectric points will

tend to remain in the cathode chamber longer, and are therefore more easily removed. Details regarding the application of the electrodialyzer for complete fractionation of the ionizable nitrogen will be presented in a separate paper. There are numerous advantages in extracting tissue in this manner, including the possibility of using colorimetric methods for estimating certain fractions.

AQUEOUS EXTRACTION OF PLANT TISSUE.—The fresh or frozen tissue was extracted with water and freed of protein by coagulation with dilute acetic acid after the method of CHIBNALL (**4, 5**).

The methods used for analysis of extracts have already been described (**39**).

Presentation of results

EXPERIMENT I.—Tomato plants were washed free of soil and set in sand when 4 inches high. They were given a minus-nitrogen solution until the stems were stiff and woody and the leaves yellowish green. Starch was abundant in all parenchymatous tissue. The plants were then divided into two series of twenty cultures each. One series was supplied with ammonium sulphate in a complete nutrient solution at pH 8.0, the other sodium nitrate at pH 5.1. The solution was not held at a constant pH (**38**). The roots, stems, and leaves were analyzed for nitrogenous fractions. Figure 1*a* shows these plants 3 weeks after being given nutrient solutions B and C (table I). Three weeks later, on October 15, when the plants of both series were about 10 inches high, they were harvested and analyzed. The chemical data are shown in table II and in columns 1 and 2 of figure 2.

The ammonium-supplied plants were definitely succulent, and had in the upper stem a very active cambium and starch (only in the region near the phloem). In the base of the stem there was still some starch in the pith. The tap root contained starch only in the endodermis. The plants which received nitrate had not become so succulent as the ammonium-supplied series and the cambium was not so active. Starch was distributed more generally throughout the plants with the exception of the tip. The dry matter of the roots and leaves, however, was only slightly higher. The plants of the ammonium series, as compared with those receiving nitrate, were more spreading, owing to softer petioles and slightly larger leaflets which had a tendency to droop more. The internodes, however, were slightly longer in the plants receiving nitrate.

EXPERIMENT II.—One lot of twelve tomato plants was grown to the fruiting stage with an abundant supply of ammonium sulphate, to determine how much organic nitrogen would accumulate, and whether it would be possible so completely to utilize carbohydrates that the plant might be injured as a result. A complete nutrient solution of which one-half of the

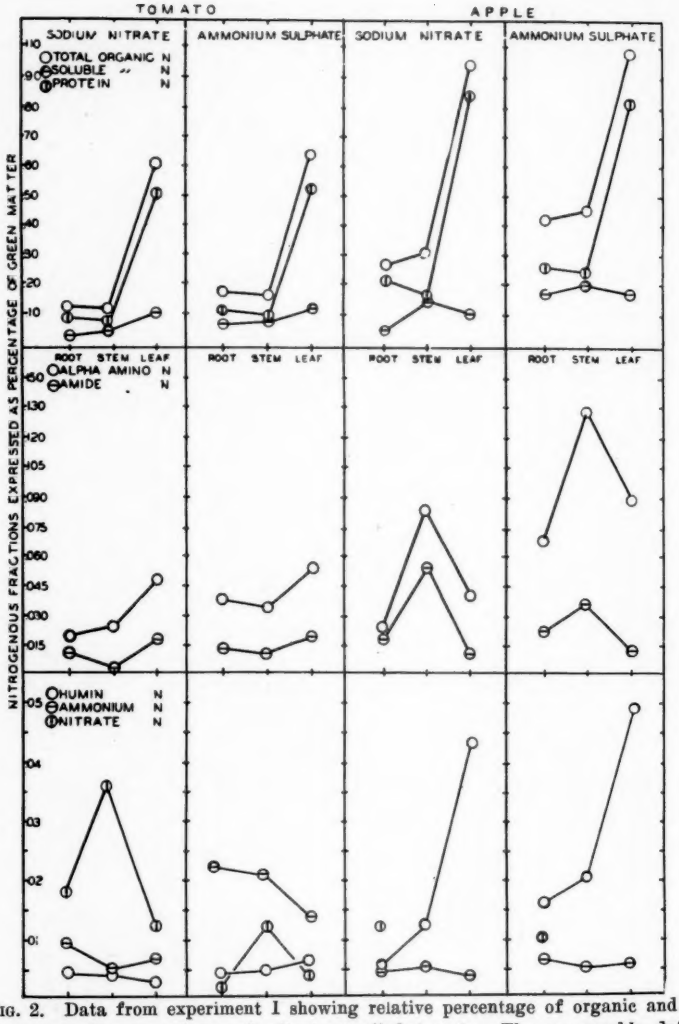

FIG. 2. Data from experiment I showing relative percentage of organic and inorganic nitrogen in ammonium and nitrate-supplied tomato. The comparable data on apple (experiment I) have been presented elsewhere (39) but are presented here for comparative purposes.

TABLE II

EXPERIMENT I

NITROGENOUS FRACTIONS IN CARBOHYDRATE-HIGH TOMATO PLANTS SIX WEEKS AFTER BEING SUPPLIED WITH SODIUM NITRATE AT pH 5.1 OR AMMONIUM HYDROXIDE AT pH 8.0

	NITROGEN AS PERCENTAGE OF GREEN MATTER						PERCENTAGE OF TOTAL ORGANIC NITROGEN					
	ROOTS		STEMS		LEAVES		ROOTS		STEMS		LEAVES	
	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3
	%	%	%	%	%	%	%	%	%	%	%	%
Percentage dry matter	8.0	6.0	7.0	8.0	13.0	12.0	.....	.....	.....	.....	.....	.....
Total organic N	0.1217	0.1754	0.1187	0.1631	0.6040	0.6420	100.00	100.00	100.00	100.00	100.00	100.00
Protein N	0.0856	0.1095	0.0728	0.0919	0.5055	0.5255	70.34	62.43	61.33	56.35	83.69	81.85
Soluble organic N	0.0361	0.0659	0.0459	0.0712	0.0985	0.1165	29.66	37.57	38.67	43.65	16.31	18.15
Humin N	0.0045	0.0040	0.0037	0.0048	0.0025	0.0060	3.70	2.28	3.12	2.94	0.41	0.93
α Amino N	0.0162	0.0387	0.0221	0.0375	0.0443	0.0544	13.31	22.06	18.62	22.99	7.33	8.47
Amide N	0.0083	0.0130	0.0017	0.0118	0.0110	0.0194	6.82	7.41	1.43	7.23	1.82	3.02
Ammonium N*	0.0092	0.0221	0.0059	0.0218	0.0075	0.0135	6.32	11.07	3.68	11.03	1.20	2.05
Nitrate N†	0.0147	0.0022	0.0357	0.0127	0.0125	0.0045	10.10	1.10	21.60	6.43	2.00	0.68
Other N	0.0071	0.0102	0.0184	0.0171	0.0407	0.0367	5.83	5.82	15.50	10.49	6.75	5.72

* Percentage of total nitrogen.

† Percentage of total nitrogen (includes nitrate determined in extract from electrodialyzed tissue; none was recovered from ammonium plants by ordinary extraction).

TABLE III

Experiment II

Nitrogenous fractions as percentage of green matter in different parts of tomato plants which received ammonium sulphate as the only source of nitrogen in a complete nutrient solution supplied at initial pH 8.0 and final pH 3.8

	Plants harvested December 11											
	Fibrous roots		Stems		Old blades		Young blades		Old petioles		Young petioles	
	%	%	%	%	%	%	%	%	%	%	%	%
Percentage dry matter	9.68	.....	10.13	.....	9.2	.....	10.4	.....	7.03	.....	6.4	.....
Total organic N	0.4800	100.00	0.3765	100.00	0.4230	100.00	0.6641	100.00	0.2171	100.00	0.2162	100.00
Protein N	0.2490	51.88	0.1443	38.33	0.2818	66.62	0.5003	75.34	0.0978	45.05	0.1045	48.34
Soluble organic N	0.2310	48.12	0.2322	61.67	0.1412	33.38	0.1638	24.66	0.1193	54.95	0.1117	51.66
Basic N	0.0296	6.17	0.0200	5.31	0.0405	9.57	0.0390	5.87	0.0195	8.98	0.0146	6.75
α Amino N	0.1211	25.23	0.1220	32.40	0.0561	13.26	0.0665	10.01	0.0617	28.42	0.0561	25.95
Amide N	0.0416	8.67	0.0275	7.30	0.0175	4.14	0.0190	2.86	0.0240	11.05	0.0346	16.00
Ammonium N*	0.0690	12.34	0.0450	10.55	0.0730	14.46	0.0550	7.58	0.0670	22.90	0.0430	16.21
Nitrate N†	0.0103	1.84	0.0050	1.17	0.0090	1.78	0.0065	0.90	0.0085	2.90	0.0061	2.30
Other N	0.0387	8.06	0.0627	16.65	0.0271	6.45	0.0393	5.92	0.0141	6.49	0.0064	2.96

* A large amount of this ammonia is combined ammonia (40).

† Ammonia and nitrate expressed as percentage of total nitrogen.

concentration was ammonium sulphate (E, table I) was supplied at pH 8. The ammonium sulphate was gradually increased from solution A to E as the plants grew larger. The plants were harvested December 11, at which time the days were short and relatively cloudy. The night temperature of the greenhouse during this period was kept between 56° and 62° F.

The plants were extremely succulent, although they grew slowly, and the leaves contained only 10 per cent. dry matter and practically no starch. The stems were more or less hollow, owing to disintegration of pith cells, and were comparatively soft. The plants were unfruitful, although they produced occasional flowers. There was no external indication of the high percentage of ammonium present. A trace of nitrate was detected throughout the plant. The nitrogenous fractions in the different plant parts are shown in table III.

EXPERIMENT III.—From April 20 to August 15, two series each consisting of twenty 1-year-old Delicious apple trees were grown in sand cultures. They were supplied with sodium nitrate at initial pH 5.1, and ammonium hydroxide at initial pH 8 (B and D respectively, table I). The chemical data and observations are reported in detail elsewhere (**39**), but for comparative purposes are summarized graphically in figure 2, columns 3 and 4.

EXPERIMENT IV.—One-year-old Delicious apple trees were set in nitrogen-free sand and grown with minus-nitrogen solution until all the inorganic nitrogen in the plants had been assimilated. They made from 12 to 15 inches of stem growth, the leaves became yellow and eventually abscissed, and the trees become dormant. These dormant trees were divided into two series of twenty each, were supplied daily with nitrate or ammonium solutions (B and D, table I), and were analyzed at intervals to determine the relative rates of assimilation of the two forms of nitrogen in dormant trees. The results have been published (**39**) but will be referred to in the discussion.

EXPERIMENT V.—One-year-old Delicious apple trees were placed in sand cultures and supplied with nutrient solutions containing ammonium sulphate or sodium nitrate at initial pH values ranging from 3.5 to 8.5 (B and C, table I). Ten trees were grown at each pH value. Detailed results have been reported (**39**), but are summarized graphically in figure 3.

EXPERIMENT VI.—In order to determine how the results obtained with the tomato in sand cultures would apply to soil conditions, a sandy loam soil was selected with a reaction of pH 4.0. Lime was added to two lots of this soil, so that three series were established; the original, pH 4.0, and the two limed series, 6.0 and 7.4. Tomato plants high in carbohydrates, with stiff woody stems and small yellowish leaves, were planted in each soil series (twenty plants at each pH) and supplied with nutrient solutions containing sodium nitrate, ammonium sulphate, and ammonium hydroxide re-

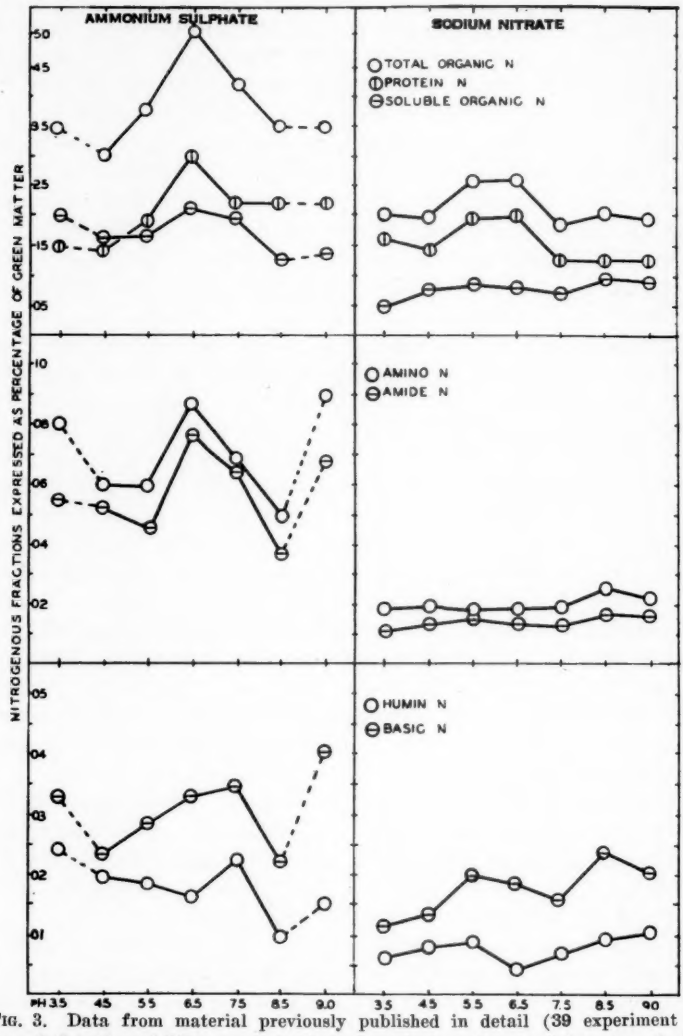

FIG. 3. Data from material previously published in detail (39 experiment III) showing relationships of H-ion concentration of nutrient medium to elaboration of nitrogenous fractions in roots of apple trees supplied with sodium nitrate or ammonium sulphate.

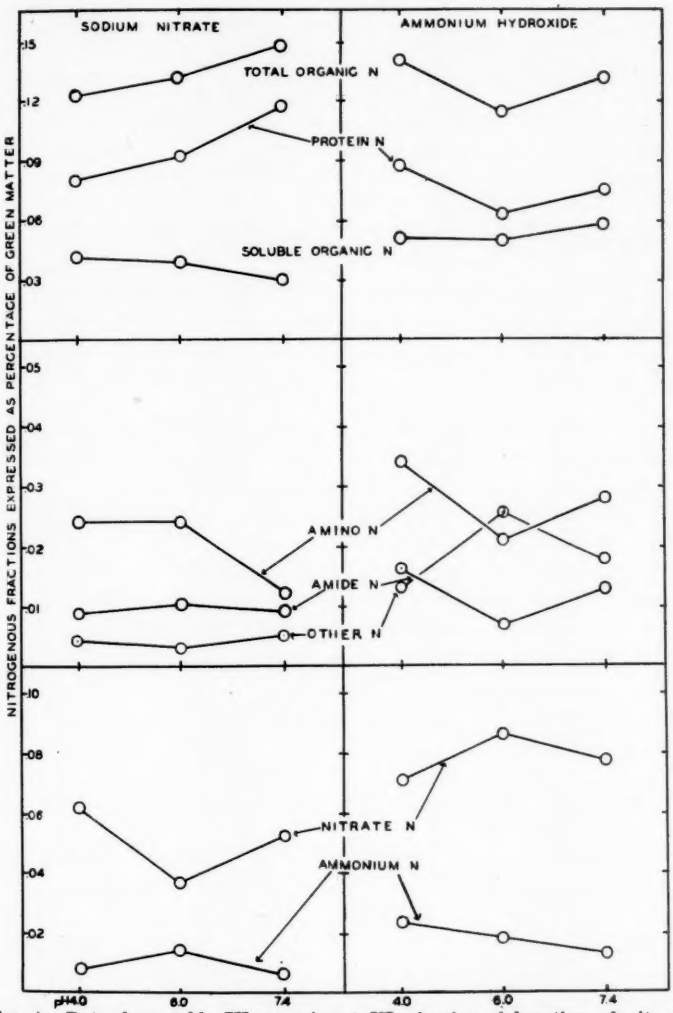

FIG. 4. Data from table IV, experiment VI, showing elaboration of nitrogenous fractions in stems of tomato plants grown on a soil adjusted to pH 4, 6.0, and 7.4 with hydrated lime and supplied with nutrient solution containing sodium nitrate or ammonium hydroxide.

TABLE IV

EXPERIMENT VI

TOMATO PLANTS GROWN IN SOIL ADJUSTED WITH HYDRATED LIME TO THE H-ION CONCENTRATION INDICATED AND SUPPLIED WITH NITRATE OF SODA OR AMMONIUM HYDROXIDE

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

Source of nitrogen	$NaNO_3$	$NaNO_3$	$NaNO_3$	NH_4OH	NH_4OH	NH_4OH	None
Dry matter (%)	6.5	6.8	6.5	5.6	6.0	6.3	7.7
pH of soil	4.0	6.0	7.4	4.0	6.0	7.4	4.0
Organic N	0.1226	0.1330	0.1473	0.1396	0.1137	0.1317	0.1214
Protein N	0.0800	0.0917	0.1193	0.0876	0.0618	0.0753	0.0671
Soluble organic N	0.0426	0.0413	0.0300	0.0520	0.0519	0.0564	0.0543
α Amino N	0.0239	0.0241	0.0125	0.0338	0.0211	0.0280	0.0324
Amide N	0.0098	0.0113	0.0093	0.0152	0.0071	0.0129	0.0235
Ammonium N	0.0082	0.0141	0.0066	0.0232	0.0178	0.0133	0.0000
Nitrate N	0.0615	0.0352	0.0525	0.0710	0.0851	0.0775	0.0000
Other N	0.0089	0.0059	0.0092	0.0130	0.0237	0.0165	0.0016

spectively. Plants when harvested (for analysis, table IV, figure 4) were 12–14 inches high; the remainder were grown to maturity. Here again the ammonium-supplied plants on the soil having a pH value of 7.4 were largest. At pH 4.0, plants supplied with ammonium sulphate made very poor growth. Microchemical tests for nitrate in the plants of the ammonium-supplied cultures showed only a trace as compared with abundant nitrates in the plants which received sodium nitrate at the same pH. Those in the cultures supplied with ammonium hydroxide at pH 4.0 did not make much growth until nitrification occurred in the soil. The plants in the nitrate cultures, in contrast to the ammonium group, made the most rapid growth at pH 4. Growth of the plants in the pH 6.0 and 7.4 cultures receiving ammonium nitrogen made a greater volume of growth than those receiving nitrate nitrogen at the same pH. The organic nitrogenous fractions are similar to those found in tomato plants grown in sand cultures with single sources of nitrogen (**40**).

EXPERIMENT VII.—During November and December, accompanied by almost continuously cloudy conditions, two series of tomato plants were grown in sand cultures at a night temperature of 58°–62° F. On occasional bright days the temperature was 5°–10° higher. One group received sodium nitrate in complete nutrient solution at an initial pH of 5.1, while the other received ammonium sulphate plus ammonium hydroxide in a complete nutrient solution at an initial pH of 8. Nutrient solutions with one-half of their concentrations made up with the nitrogenous salts (B and C, table I) were supplied to different groups of nine plants each at concentrations of approximately 1.50, 1.25, 1.00, 0.75, 0.50, and 0.25 atmospheres. Another lot of nutrient solutions (F and G, table I) were made up so that

only one-fifth of their concentration was made up by the nitrogenous salts. These nutrient solutions were supplied to different groups of nine plants each at concentrations of approximately 1.00 0.75, 0.50, and 0.25 atmospheres. Thus different groups of plants had available concentrations of nitrogen varying from extremely high to extremely low.

The plants growing with sodium nitrate made their greatest volume of growth with the highest concentration of nitrate, and the volume of growth decreased as the concentration of nitrate in the nutrient solution and in the tissue decreased. Microchemical observations showed that starch increased in the stems as the nitrate concentration decreased, but reducing sugars did not show a definite correlation with growth.

The plants of the ammonium cultures made the greatest volume of growth with the 0.50-atmosphere concentration nutrient solution containing a low concentration of ammonium salt. The two cultures receiving the higher concentrations of ammonium salt, at 1.5 and 1.25 atmospheres, made very little growth and remained grayish green, although the plants including the roots appeared uninjured. Only the stems of the ammonium-supplied plants which were growing rapidly contained starch. As the ammonium concentration in the nutrient solution increased, growth was retarded, accompanied by a deficiency of carbohydrates. Only a trace of starch could be detected in the plants supplied with the high concentration of ammonium, and that was in the endodermis. The stems of the plants tended to be hollow, and the cell walls of the xylem tissue were thinner than any of the nitrate-supplied plants.

EXPERIMENT VIII.—An experiment with Delicious apple trees similar to experiment VII with tomatoes, but conducted from January 1 to April 1, resulted in observations of a comparable nature. The trees with different concentrations of nitrate nitrogen grew fairly well (fig. 5), but growth was most rapid at one atmosphere concentration. The trees receiving the extremely low concentration of ammonium nitrogen were equal to the nitrate trees, but very little growth occurred with the high concentration of ammonium.

EXPERIMENT IX.—Tomato and cotton plants, sweet clover seedlings, and Delicious, Stayman, and Baldwin apple trees were grown in sand cultures in which the pH of the nutrient solution dripping through the sand did not increase nor decrease more than 0.2 pH from that initially supplied. Solution B (table I) was used for the nitrate cultures and solution F (table I) for the ammonium series. From 4 to 12 liters of solution, depending on the size of the plants, were dripped through sand of the individual crocks every 12 hours. Sufficient cultures were set up to give cultures from pH 3.5 to 6.5 at intervals of 0.5 pH.

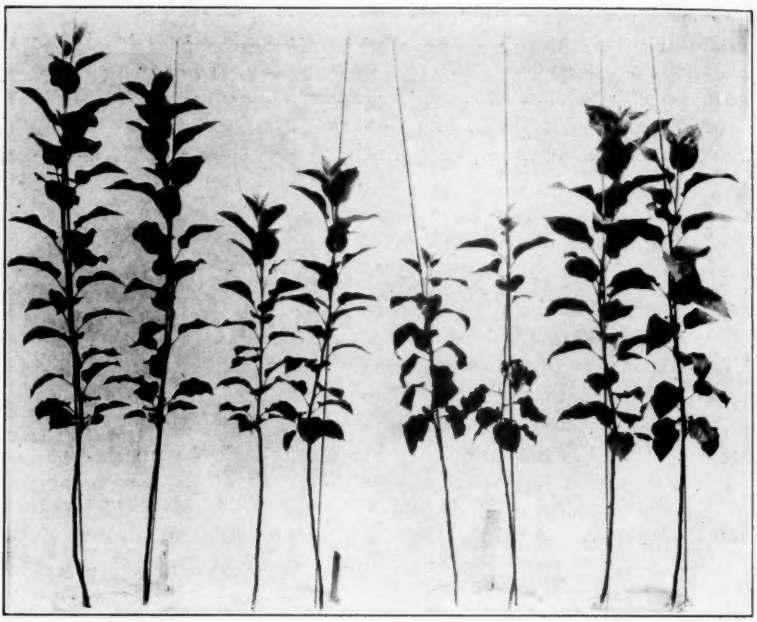

FIG. 5. Delicious apple trees of experiment VIII grown in sand cultures with equivalent concentrations of nitrate and ammonium nitrogen. From left to right, culture no. 1 received a high and no. 2 a low concentration of sodium nitrate, and no. 3 a high and no. 4 a low concentration of ammonium sulphate in the nutrient solution.

The tomato and cotton plants and sweet clover seedlings assimilated ammonium most satisfactorily (made the greatest volume of green growth) at pH 6.0 and nitrate at pH 4.0. The apple varieties assimilated ammonium nitrogen most satisfactorily at pH 6.5 and nitrate at pH 4.5. The Baldwin and Stayman trees made considerable top growth and fair root growth with ammonium at pH 4.0. The Delicious trees, however, made no root growth at 4.0 and only a fair growth at pH 5.0 with ammonium nitrogen. In a later experiment it was found that if the Delicious trees were supplied with a low concentration of ammonium (0.0014 p.v.m.) the roots grew slowly although the trees did not grow. A high concentration of ammonium retarded root growth more in the Delicious than in the other two varieties.

These results are interesting because they were corroborated in the field on Rome trees growing on sandy soils of New Jersey having a reaction of pH 3.7. Calcium nitrate stimulated root growth and top growth but ammonium nitrogen did not stimulate growth. When some of this same soil

was taken into the greenhouse it was found that the trees did not respond to ammonium sulphate unless at least the equivalent of two tons of hydrated lime to the acre had been added. Sodium nitrate did not give a response unless calcium was supplied. It seemed fully as important to supply calcium in the field as in the sand cultures in order that good root growth and nitrate assimilation would take place. It was not necessary to change the reaction of the soil; however, the addition of calcium was not sufficient to cause ammonium to be assimilated. The reaction of the soil had to be increased to a higher pH value before the trees were stimulated by the ammonium. Incidentally, nitrification of the ammonium became more rapid as the pH of the soil was increased.

Discussion

The pH value of nutrient solution

Results from sand-culture experiments have a broader application to the growth of plants in their natural habitats if the pH of the culture can be maintained at a constant value. The pH value of a soil changes only slightly from year to year. Previous results on the growth of the tomato (**40**) and the apple (**39**) suggest that the pH value of the nutrient solution must be comparatively high for satisfactory assimilation of ammonium nitrogen. This may be misleading unless the pH values of the solutions, before and after dripping through the sand, are kept in mind.

When a nutrient solution containing nitrogen only as ammonium is supplied to a mature tomato plant at pH 8.0 and comes out of the culture at pH 4.0, as in the work already reported (**39, 40**), the roots of necessity are bathed in a nutrient solution of considerable variation, although the growth of the plant is very luxuriant, indicating rapid assimilation of the ammonium ion. If the initial pH of the solution is lower, growth is less luxuriant. In experiment IX it was shown that if the nutrient solution containing nitrogen only as ammonium was supplied at pH 6.0, and added in sufficient quantity to prevent a drop greater than 0.2 pH in 24 hours, growth was luxuriant. The growth at a practically constant pH of 6.0 was comparable with that produced by plants grown in a solution changing from pH 8.0 to 4.0 (**40**). This seems reasonable because the composite pH value of the variable culture was about 6.0. Similar responses to pH values were observed with cotton (**38**) and three varieties of apples (**39**). In sand culture, rapid renewal of the nutrient solution is necessary to prevent the pH from dropping materially. Results of hydrogen-ion concentration experiments previously published (**38, 39, 40**), therefore, should be interpreted on the basis of intermediate values between the initial pH of the nutrient solution and the pH after passing through the sand. The

results would then be essentially comparable with the experiments in which the nutrient solution was maintained at an approximately constant pH.

Tomato, cotton, and apple with ammonium made very slow growth at a constant pH value of 8.0, more rapid at 7.0, and most rapid at 6.0 Nutrient solutions containing nitrogen as ammonium only gave unsatisfactory results unless a constant pH of 5.0 to 6.0 was maintained. Whether trees can assimilate ammonium and make rapid growth depends on the ammonium concentration of the nutrient solution and the carbohydrate reserves in the stem. Perhaps because of the carbohydrate reserves in woody plants, ammonium may be assimilated more rapidly over a wider range of pH values than would be true of herbaceous plants. Fluctuating concentrations of ammonium are also less noticeable in the resultant growth of woody plants because of the greater supply of carbohydrates. Plants supplied with complete nutrient solutions containing nitrogen only as nitrate tended to absorb both cations and anions, so that a residual pH of 6.0 was approximated (**40**). If the nitrate was substituted by ammonium, however, the residual pH did not come to equilibrium at 6.0, but decreased to pH values as low as 3.8 (**40**). These residual changes in the nutrient solutions occurred only when plants were assimilating nitrate or ammonium nitrogen rapidly.

The absorption of ions from a solution containing nitrate apparently depends on the effect of the pH of the nutrient solution on the rate of assimilation of the nitrate or ammonium ion, or on the growth rate of the plants (**40**). The absorption of the nitrate ion decreases anions more rapidly than cations at pH 4.0, and equilibrium is maintained at approximately pH 6.0. If the pH is high enough to bring about assimilation, solutions containing ammonium nitrogen do not come to equilibrium but become increasingly more acid, owing to the residual anions and the rapidity with which ammonium is assimilated. The ammonium ion is absorbed more rapidly than the anions. This increase in acidity depends on the rate of absorption of ammonium, which in turn depends on whether the ammonium ion is assimilated as rapidly as it is absorbed from the nutrient solution. If the ammonium ion accumulates in the cell and absorption ceases, the acidity of the nutrient solution does not increase as rapidly because the residual anions do not accumulate. This may cause the residual solution to come to equilibrium at a pH value comparable with that when the nitrate ion is present. Figure 6 shows that accumulation of the ammonium ion in the plant is directly or indirectly a function of the pH of the nutrient solution. Thus it is only those plants that are assimilating ammonium rapidly which maintain a rapid absorption of ammonium ions. If the cells become saturated with ammonium ions, they can no longer absorb more.

The best growth is made with nitrate nitrogen at pH 4.0, whether the

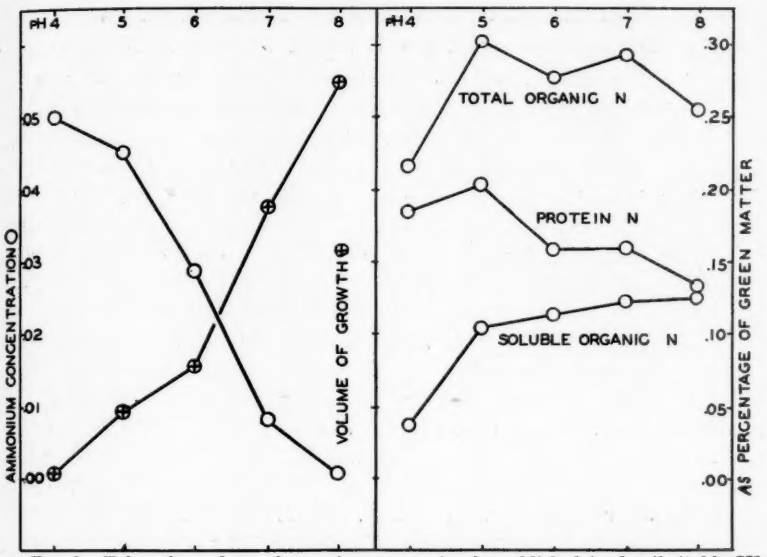

FIG. 6. Taken from data of experiments previously published in detail (table IV, 40) showing relationships between volume of growth (size of green plants in gm.) of tomato plants when supplied with nutrient solutions having different initial pH values and accumulation of ammonium in stems. Rate of assimilation of ammonia is directly correlated with growth.

pH of the residual solution remains constant or tends to come to equilibrium (**39, 40**). The pH values at which nitrate or ammonium nitrogen are satisfactorily assimilated are probably characteristic for the variety or species under observation, and are not particularly associated with the neutral point.

That plants will assimilate ammonium nitrogen satisfactorily has been demonstrated (**40**). PIRSCHLE (**29**) listed a great number of economic plants that make a rapid growth with ammonium nitrogen, and demonstrated that the pH of the nutrient solution had a controlling influence on its assimilation. He found that tobacco produced twice as much total dry matter with ammonium sulphate as with sodium nitrate at pH 6.0, although BEAUMONT *et al.* (**2**) state that tobacco does not assimilate ammonium satisfactorily. THOMAS (**36**) also states that ammonium is not satisfactorily assimilated in tobacco, and DAVIS (**7**) states that it is not assimilated in the apple; but in all instances the pH of the nutrient solutions may have been at fault, as has been demonstrated (**21, 29, 39, 40**). The pH of the nutrient solution, therefore, has directly or indirectly a controlling influence on the assimilation, particularly of the ammonium ion, and somewhat less pronounced of the nitrate ion.

Assimilation of ammonium and nitrate nitrogen

Eckerson (**8**) and others (**25, 26**) have found that plants, through the action of a nitrate-reducing material (reducase), oxidize carbohydrates or organic acids, and reduce nitrates to nitrites and ammonium, following which there are synthesized amino acids and other organic nitrogenous material. Reducase activity, however, may be affected by length of day, light intensity, or other external factors (**9, 11**), all of which will tend to modify the rate of assimilation of nitrate nitrogen (**11**). The utilization of absorbed ammonium by the tomato plants and apple trees in the experiments reported here was apparently rapid, and did not seem to be checked by lack of reducase as may possibly have been the case in the nitrate-supplied plants. This assumption would seem to be supported by the fact that amino acids increased more rapidly and in greater quantities in plants supplied with ammonium than in those supplied with nitrate nitrogen. This is shown by the data in tables II and IV for tomato and in figures 2 and 3 for apple. Likewise in 3 days the ratio of protein to non-protein nitrogen (**39**) in dormant apple trees changed from 73 : 27 to 61 : 39 in those supplied with nitrate, and to 56 : 44 in the trees of the ammonium series. Accompanying this change there was an 18 per cent. increase in total organic nitrogen in the roots of the trees receiving ammonium. Furthermore the maximum increase in total organic nitrogen in the trees supplied with ammonium occurred in 35 days, as compared with 56 days for the trees supplied with nitrate nitrogen. That a similar condition exists in the tomato is shown in table II and figure 6. Thus the general idea that ammonium is more slowly available than nitrate is not borne out by these experiments, but supports the contention (**37**) that ammonium may be as quickly available as nitrate nitrogen.

Eckerson (**9, 10, 11**) finds that some plants, including the tomato, but especially soy bean, have comparatively weak reducase activity during the short days of winter, and that carbohydrates may accumulate in the presence of abundant nitrate. It would seem, therefore, that with synthesis of organic nitrogen from ammonium there should be more rapid decrease in carbohydrates than with nitrate nitrogen under similar conditions. This is apparently borne out, at least for tomato, by figure 1 *a*, *b*. The relatively rapid decrease of starch and reducing sugars in apple roots supplied with ammonium as compared with nitrate nitrogen further emphasizes this assumption (**39**). Benecke (**3**) has suggested a similar relationship.

Observations and microchemical tests of the tomato plants of experiment VII showed definitely that during short days carbohydrates decreased as ammonium concentration increased. Nitrate-supplied plants showed con-

siderable starch, however, even at the highest concentration of nitrate nitrogen. Thus it would seem that, in order to obtain a comparable growth with ammonium and nitrate nitrogen, it is necessary to supply only as much ammonium to a culture as is equal to that made available by the reduction of nitrate in the cells of a plant growing in a nitrate supplied culture. This is supported by data presented by PIRSCHLE[3] (**29**). He shows that much less ammonium than nitrate nitrogen is necessary for the production of a given amount of growth.

Data by NIGHTINGALE *et al.* (**27**) showed that during comparatively long days in April the proportion of protein to soluble organic nitrogen was 52 to 48 per cent. in stems of highly vegetative non-fruitful tomato plants, under conditions which were favorable for both reducase (**10, 11**) and photosynthetic activity. This may be compared with the data on the ammonium-supplied tomato plants shown in table III, which were grown during the winter months. Here the ratio of protein to soluble organic nitrogen in the stems was 38 to 62 per cent. Thus ammonium nitrogen increased the soluble nitrogenous fraction in the tomato almost as rapidly during short winter days, when reducase activity was somewhat low, as nitrate nitrogen did under conditions favorable for abundant reducase activity. The result was a rapid utilization of carbohydrates in amino acid synthesis and extremely soft succulent growth. The stems of the tomato plants grown by NIGHTINGALE (**27**) contained 11–14 per cent. dry matter and were described as soft and succulent, yet plants of these experiments had only 10 per cent. of dry material in the stems. Under favorable environmental conditions, there seems to be no way to prevent extremely rapid utilization of carbohydrates in ammonium-supplied tomato plants or apple trees, except by limiting the external supply of ammonium.

Lack of carbohydrates does not favor condensation of amino acid to proteins (**27, 33**). It has been shown that in plants supplied with high concentrations of ammonium nitrogen (**13, 39, 40**) the proportion of complex protein nitrogen decreased.

The data on reducase show a paucity of activity in apple trees supplied with ammonium nitrogen (**39**). Apparently the synthesis of reducase did not occur, or it was inactive in the presence of appreciable quantities of ammonium. In nitrate-supplied tomato plants the rate of reducase activity seemed to prevent excessively rapid utilization of carbohydrates. In am-

[3] In the H-ion concentration studies by PIRSCHLE, it should be pointed out that many data were obtained from young plants, which have a more alkaline composite pH than mature plants. Also the concentration of ammonium nitrogen was comparatively high, so that sugars were used more rapidly than in the nitrate-supplied plants. Many of the plants which received ammonium might have synthesized as much dry matter as the plants grown with nitrate if the concentration of ammonium had been less.

monium-supplied apple trees, however, it is possible to deplete carbohydrates to the point where growth and carbohydrate storage cannot keep pace with elaboration of nitrogenous compounds. Even trees supplied with nitrate nitrogen may show signs of carbohydrate deficiency, but in a much less marked degree. This has been observed, but only during dull cloudy weather of midwinter when day length was at a minimum, whereas with ammonium nutrition it may occur in midsummer. These results are in indirect agreement with those obtained by MEVIUS and DIKUSSAR (**18**). They found that nitrites properly supplied were more rapidly assimilated than nitrates.

ORGANS CONCERNED IN ASSIMILATION OF NITRATE AND AMMONIUM NITROGEN IN TOMATO AND APPLE

NIGHTINGALE (**27**) showed that nitrate may be found throughout the entire tomato plant. It has likewise been shown (**40**) that ammonium may be traced in quantity even to the leaves of the tomato plant. The data in table III show that ammonium constitutes 7.58 per cent. of the total nitrogen in the young blades and 14.46 per cent. in the old blades. Thus it seems reasonable to suppose that the assimilation of nitrate and ammonium nitrogen occurs at least to some extent in all parts of the tomato.

NIGHTINGALE (**22**) suggests that the fine roots of pear seedlings and blackberry bushes reduce nitrates and synthesize amino acids in the roots. KRAYBILL (**14**) and THOMAS (**35**) state that a similar condition exists in the apple. The chemical data on nitrate reduction previously presented (**39**), and the fact that there were no nitrates found in the stems and leaves of the apple trees, substantiate these observations. ECKERSON, however, found a trace of nitrate in the terminal buds of the 1-year-old apple trees of experiment V. The initial pH of the nutrient solution in which these trees were grown was 8 to 9, a value which was much too high for efficient assimilation of nitrate in tomato (**40**) and the apple (**39**). Nitrate may likewise be found in the tops of other plants which usually contain nitrates in the roots only, if growth conditions are unfavorable (**25, 26**).

An appreciable concentration of ammonium was recovered in the stems and leaves of the apple (**39**). This may indicate that some ammonium is assimilated in all parts of the tree. This ammonium in the upper part of the tree was probably the result of proteolysis (**27**), however, rather than of absorption and translocation. The distribution of ammonium is therefore no proof that ammonium assimilation and the reduction of nitrates are not chiefly root processes except in unusual cases, as has been pointed out. More ammonium was found in the woody stems and leaves of the apple (**39**) than in the stems and leaves of the tomato (**40**) when these plants were grown during the summer with ammonium nitrogen.

The data of experiment IV (**39**) show amino acids to be 0.132 per cent. on a green-weight basis in the stem of the ammonium-supplied trees, as compared with only 0.078 in the nitrate series. A similar comparison may be made in the leaves and both may be largely accounted for by translocation of amino acids from the roots (**39**). There occurred also an increase in dry matter in the roots, resulting from translocation of carbohydrates from the stems; and in view of the fact (**39**) that ammonium ions are almost immediately synthesized to amino acids in the presence of carbohydrates (3 days), it is probable that the larger part of the elaboration of amino acids from both ammonium and nitrate takes place in the roots. That this also is at least partly true for relatively complex organic nitrofractions is indicated by the increase in humin and "other" nitrogen.

That the nitrate ion does not go beyond the fine roots in the apple may be due to its combination with the protoplasm (ampholytes) in the root hairs. Adsorption is particularly favored when the nutrient solution has a low pH (4.5) value, and less favored in a nutrient solution at a high pH (7.5) value. This may account for the fact that ECKERSON found nitrate in the tops of trees grown in the more alkaline nutrient solution (**39**).

EFFECTS OF pH OF NUTRIENT MEDIUM ON ELABORATION OF NITROGENOUS FRACTIONS

In figure 3 there are recorded nitrogenous fractions in the roots of apple trees which were supplied with ammonium sulphate or sodium nitrate. These data are taken from previously published tables (**39**). The greater total amount of elaborated nitrogen in the ammonium as compared with the nitrate-supplied trees is evident. This is probably due to more rapid elaboration because of the greater amount of ammonium available when directly supplied than to that obtained through nitrate reduction. The greatest difference in organic nitrogen occurred when the nutrient solution of both lots of trees was supplied with an initial pH of 6.5. The greatest volume of growth of the ammonium trees, however, occurred at pH 6.5 to 8.5. This increase in total elaborated nitrogen is accounted for by a greater proportion of protein, as well as by an increase in soluble organic nitrogen. Where the volume of growth was largest (at initial pH 7.5), the protein and soluble organic nitrogen fractions are almost equal. The greatest volume of growth in the nitrate plants occurred at pH 4.5 to 5.5, whereas total elaborated nitrogen was highest at pH 5.5 to 6.5. Although the protein fractions in the ammonium and nitrate-grown trees were more or less the same in total amount, the various determined soluble nitrogenous fractions were much higher in the ammonium-supplied plants. These data are more or less in agreement with those previously presented for tomato and soy bean (**40**).

In the nitrate-supplied trees the effect of pH of the nutrient solution on the soluble organic nitrogen fraction of the plant is negligible except at the extreme pH values. At pH 3.5 and 9.0, at which the plants made comparatively poor growth and slight root injury was apparent, there was an increase in the soluble nitrogen fraction, possibly proteolytic (**23**). The amount of growth made by the ammonium-supplied trees follows rather generally the curves for soluble organic fractions of nitrogen, providing the data from the trees grown at pH 3.5 and 9.0 are omitted for the reasons indicated.

PRIANISCHNIKOW (**30**) emphasizes the amide nitrogen fraction in plants, and concludes that asparagine probably is a readily available reserve. He further concludes that it is elaborated when an abundance of ammonium nitrogen becomes suddenly available. MOTHES (**20**) and NIGHTINGALE (**23**), however, show that asparagine likewise increases rapidly in the dark, owing to proteolysis, and decreases in the light. This fraction is very low in the tomato (**40**), except at certain pH values, and it is probable that these exceptions in the tomato are due to "combined" ammonium (**40**) nitrogen rather than to nitrogen in the amide form. Further evidence of this will be given in a later report.

EFFECTS OF SOIL PH ON ASSIMILATION OF AMMONIUM AND NITRATE NITROGEN IN TOMATO

Data in figure 4 show that on pH 6.0 and 7.4 sandy loam soils where ammonium nitrogen may be nitrified, the tomato absorbed rather large quantities of ammonium nitrogen, and that at these pH values the plants grew similarly to tomatoes in sand culture supplied with ammonium nitrogen at pH 7.5 to 8.5. It must be remembered that the soil cultures maintained a constant pH as compared with a variable one for most of the sand cultures. There was a greater percentage of protein at pH 4 than at 6 or 7.4, but soluble organic nitrogen increased as the pH increased. Amino nitrogen was low and amide nitrogen was high at pH 6, but was reversed at pH 7.4.

The nitrate-supplied plants made their greatest volume of growth at pH 4, which was accompanied by the largest percentage of soluble organic nitrogen in their tissues but the lowest percentage of protein. Amide nitrogen was low and amino nitrogen was high in the pH 6.0 and 7.4 cultures. Nitrate nitrogen was high in the pH 4.0 cultures, probably because of increased adsorption (**40**). The ammonium content in the nitrate-supplied plants at all pH values was low because conditions were favorable for nitrate assimilation. At pH 6 protein increased slightly in the nitrate cultures and the plants showed a slightly less succulent condition, which

was particularly emphasized at pH 7.4, where protein nitrogen was high and soluble organic nitrogen was low.

In spite of the fact that the ammonium-supplied plants were comparable in growth with plants grown in sand cultures with only ammonium nitrogen, they contained rather high fractions of nitrate nitrogen; in fact, much higher than the sand-culture plants supplied with nitrate at any pH value. The lower percentage of nitrate at pH 4.0 and high concentration of ammonium and organic nitrogen suggest nitrate assimilation. Furthermore these plants contained the least dry matter of any of the soil-grown plants, 5.6 per cent.

In the plants of the ammonium-supplied cultures at pH 6.0 and 7.4, however, ammonium was low and organic nitrogen high, as for tomato in sand cultures (**40**), indicating at these pH values efficient assimilation of ammonium and less efficient assimilation of the nitrate ion. In general the fractions of soluble organic nitrogen also follow closely in trend those previously pointed out for tomato plants in sand culture under comparable pH values of the nutrient medium. There would seem to be little doubt that the ammonium-supplied plants grown in soil produced a large part of their growth with ammonium nitrogen in spite of the absorbed nitrate nitrogen which tended to accumulate.

Summary

1. It is necessary that the limitations of the use of nitrogenous salts be determined before a fair comparison of their relative merits as plant nutrients can be made. Any of the following comparisons made between ammonium and nitrate nitrogen are on the assumption that the respective salts are supplied to the plants at the pH values of the nutrient medium or soil which are optimum for the assimilation of the particular ion under consideration.

2. The hydrogen-ion concentration of the nutrient medium directly or indirectly had a controlling influence on the assimilation, particularly of the ammonium ion. The nitrate ion was assimilated most satisfactorily in tomato and apple when absorbed from an acid nutrient solution of approximately pH 4.0. The ammonium ion was assimilated most satisfactorily when absorbed from a nutrient solution having a constant pH value of 5.0 to 6.5, varying somewhat for the variety.

3. Ammonium ions were immediately absorbed by plants without further change, and were assimilated (synthesized to amino acids and other organic nitrogenous materials) directly and more rapidly than the nitrate ion.

4. Nitrate ions were apparently absorbed, reduced to nitrite, and finally to ammonium ions by the action of reducase (nitrate-reducing material in

the plant). The ammonium ion was assimilated directly and as rapidly as it was absorbed, however, whereas the nitrate ion usually tended to accumulate, at least partly, because of limited reducase activity under certain external and internal conditions (**11**). The ammonium ion was, therefore, more quickly available for the synthesis of amino acids. The ammonium ion did not accumulate in plant tissue unless the plant could not assimilate this form of nitrogen.

5. The volume of growth obtained from nitrate and ammonium depended on the concentration of the nitrogenous salt in the nutrient solution and available carbohydrates.

6. There was a direct correlation between the concentration of nitrate nitrogen and the volume of growth produced if the plant was actively reducing nitrate. Plants required a much lower concentration of ammonium than nitrate nitrogen in the nutrient solution to produce an equal volume of growth.

7. Plants containing a large amount of available carbohydrates assimilated ammonium much more rapidly than those containing a comparatively small supply of available carbohydrates. Tomato and apple grown with ammonium contained a much higher concentration of soluble organic nitrogen than those supplied with an equal quantity of nitrate. There was a direct correlation between the concentration of ammonium in the nutrient solution and the amount of soluble organic nitrogen elaborated by the tomato and apple.

8. Tomato and apple growing on soil containing ammonium and nitrate ions absorbed both ions. Whether they assimilated both ions depended partly on the pH of the soil. On acid soils having a pH of 4.0, plants tended to accumulate ammonium and assimilate nitrate ions. On neutral or slightly alkaline soils, plants tended to accumulate nitrate ions and assimilate ammonium. When ammonium salts were applied to soil cultures, some of the ammonium ions were oxidized to nitric acid and were absorbed by plants as nitrate ions.

9. In general, ammonium and nitrate salts produced equally good growth, provided their limitations were recognized. Whether nitrates were reduced in the plant to ammonium nitrogen or the nitrogenous nutrient was supplied directly as ammonium, the quality of organic nitrogen in plants grown with salts from either group was similar when the total concentration of elaborated nitrogen was the same.

10. In field experiments where comparisons are made between salts of either group, comparative data may be of little value unless the condition of the soil and the quantity of nitrogenous ions absorbed and assimilated by the plants supplied with salts from either group are specifically evaluated. If salts from the two groups are used under optimum conditions for

each, the only advantage one salt would have over the other would be brought about as a secondary effect by the ion with which the nitrate or ammonium ion was associated.

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CHLOROPHYLL PRODUCTION UNDER VARIOUS ENVIRONMENTAL CONDITIONS[1]

GUS B. ULVIN

(WITH EIGHT FIGURES)

Introduction

Chemical investigation of chlorophyll dates back to BERZELIUS, who, in 1838, treated the alcoholic extract of leaves with concentrated hydrochloric acid or alkali because he thought the leaf pigments were not decomposed by these reagents. He obtained only products of radical decomposition. Other investigators who used this same method were MULDER, 1844, and MOROT, 1849. It was believed by VERDEIL, 1851, that he could isolate pure chlorophyll by precipitating boiling alcoholic leaf extract with lime water and then treating the precipitate with hydrochloric acid. The hypothesis of the relationship between the coloring matter of leaves and of blood originated with VERDEIL (WILLSTÄTTER **11**).

Different theories have naturally been entertained about the composition of chlorophyll. As late as 1906 some thought that monocotyledons and dicotyledons did not contain the same kind of chlorophyll. Others were able to find several different kinds of chlorophyll in a single plant, and an unlimited number when derived from different plant sources. By far the greatest contribution to the chemistry of chlorophyll up to the present time was made by WILLSTÄTTER (**11**) and his collaborators. Their work covers the period from 1906 to 1913.

Leaf powder was used exclusively in the older methods of chlorophyll extraction and in rather large portions. Twelve-liter bottles were used to obtain "bottle extracts," and percolators, ranging up to 25 liters in size, were sometimes used for extracting chlorophyll. Loss of phytol ($C_{20}H_{39}OH$) by alcoholysis, and change in chlorophyll on long standing, are disadvantages in these longer methods.

The extraction method as suggested by SCHERTZ (**8**) in 1928 and used in this experiment makes use of fresh leaf material instead of dry leaf powder as formerly used. Grinding and extracting requires one-half hour or a little longer, in contrast with 24 to 48 hours used just for extracting by some of the older methods.

The purpose of this investigation was to study the quantitative production of chlorophyll under different light periods in soy beans and radishes, and in corn in the presence or absence of iron or manganese individually and in the presence or absence of both in the nutrient solution. It was

[1] Contribution from the Hull Botanical Laboratory, the University of Chicago.

thought possible to control the carbohydrate manufacture within the plant by controlling the light, as against controlling the nitrogen supply in the nutrient solution, which is the method usually adopted when it is desired to produce plants with either a high or a low carbohydrate-nitrogen ratio.

Methods

All of the plants used in these experiments were grown in greenhouses covered with ordinary glass. In experiment I, soy beans of the variety Manchu, and two varieties of radishes, Early Carmine Turnip and French Breakfast, were used. After two plantings of Early Carmine Turnip no more seed of that variety was available and the other was substituted. One set of plants was grown in 10-hour light periods, the other in continuous light. The short light period plants were taken out of the dark room at 8 A.M. and put back at 6 P.M. The plants receiving continuous light received the total natural daylight, supplemented by electric light during the remaining hours of each 24. Three 1000-watt Mazda bulbs with reflectors were used 26.5 inches above each group of plants to supplement daylight.

The light intensity was measured several times and it was found that approximately 25 per cent. of the full intensity of sunlight was lost to the plants in the greenhouse. On a very bright day the intensity of full sunlight was found to be 8436 foot candles, 5536 foot candles in the greenhouse, and only 2046 foot candles under a curtain of muslin used to shade the plants on bright days. On a slightly hazy day, sunlight had an intensity of 6775 foot candles, but was reduced in the greenhouse to 5239 foot candles. In the first case the loss in passing through one thickness of greenhouse glass was 34.37 per cent., and in the second, 22.67 per cent. The loss indicated by two other readings was 31.13 per cent. The intensity from three 1000-watt electric light bulbs varied from 650 foot candles at a distance of 26.5 inches from the bulbs when all were new, to 431 foot candles after several months' use.

A hundred or more soy beans were seeded in a pot of sand under natural conditions, and when six or seven days old, seven plants were transplanted into each of several 2-gallon glazed pots. These plants were grown in quartz sand with DAVIDSON'S (1) nutrient solution 3 T, R, C_3. This is a four-salt solution supplying nitrogen as nitrate nitrogen in calcium nitrate and as ammonium nitrogen in ammonium sulphate. One-half molar stock solutions were made up of each of the four salts and these added in proper amounts to distilled water to make the nutrient solution. A few parts per million of manganese and boron were added to the nutrient solution at each application and iron as ferric phosphate whenever needed. The radish plants required much more Fe than did the soy beans. The soy bean plants

were planted at intervals and each individual planting is referred to as series 1, 2, etc.

In experiment II, a garden variety of sweet corn, Golden Bantam, was used. A three-salt nutrient solution containing nitrogen only as nitrate nitrogen in calcium nitrate was used. The effect of the presence or absence of iron and manganese on chlorophyll production was studied. A series of four treatments was used in which one set of four pots received neither iron nor manganese; the second set received Fe 1 p.p.m. but no manganese; the third set received Fe and Mn at the rate of 1 p.p.m. each; and the fourth set received Fe 15 p.p.m. and Mn 1 p.p.m.

Chlorophyll extraction

The method of extracting and separating chlorophyll (α and β) from the other leaf pigments in green leaves, as suggested by Schertz (**8**), was adopted with slight modifications for this experiment. Percentage chlorophyll content of leaves on both green weight and dry weight basis as well as on an area basis was wanted. Material was often scarce, therefore 5-gram leaf samples were used instead of 10-gram samples as recommended by Schertz. Five-gram leaf samples were weighed out in duplicate and traced on paper as quickly as possible. One sample was placed in a refrigerator while the other sample was being ground and extracted according to the following method:

The 5-gram sample of fresh leaves, after being weighed and traced, was placed in a glass mortar with a little sodium carbonate to neutralize free acids, and ground for seven minutes in the dry mortar. Then about 25 grams of washed quartz sand were added and the grinding continued for seven minutes, when 30 cc. of pure acetone were added and the grinding continued for another seven minutes. A total of 21 minutes of grinding for each sample was found to be the shortest time in which the leaf tissue could be thoroughly disintegrated. This acetone extract was filtered through a Büchner funnel, under suction, and the residue washed with pure acetone until the filtrate was colorless. About 100 cc. were usually required for this. The residue was then washed with about 100 cc. of ether.

The combined ether-acetone filtrate was poured into a liter separatory funnel where the acetone was washed out with distilled water. Then 100-cc. portions of water were poured down the side of the separatory funnel with the aid of a small funnel. An aqueous layer, colored somewhat yellowish from the dissolved flavones, separated out below the ether. The liquids were rotated carefully and the aqueous layer drawn off. Repeating this washing three times with each sample of material used in this experiment was sufficient to make the last washing colorless; but with old apple leaves twelve or more washings were necessary.

The remainder of the flavones and anthocyanins, if present, were removed by washing the ether solution with a 1 per cent. solution of sodium carbonate. Some of the chlorophyll may be lost by this method; but if the flavones and anthocyanins are not removed, their presence results in too high a value for the chlorophylls when estimated colorimetrically. SCHERTZ (8) found that a 1 per cent. sodium carbonate solution would remove no chlorophyll from a pure chlorophyll-ether solution. He concludes that any chlorophyll removed by this method would be chlorophyll already decomposed in the leaf.

The ether solution was run into a 500-cc. bottle and 20 cc. of a colorless saturated potassium hydroxide-methyl alcohol solution added. This was shaken strongly and set in a refrigerator until the next day.

SEPARATION OF PIGMENTS

The ether-alkali-chlorophyll solution was taken from the refrigerator and poured into a liter separatory funnel, the bottle washed several times with a few cc. of distilled water and this added to the separatory funnel. One hundred cc. of ether should be added at this point, so the bottle in which the chlorophyll solution had been stored was washed with this ether. The separatory funnel was shaken strongly and allowed to stand for 20 minutes.

The chlorophyllin layer was then run off into a small separatory funnel. The ether in the large separatory funnel was washed two or three times with small quantities of water and this added to the small separatory funnel. The ether solution was washed with a few cc. of dilute potassium hydroxide solution, and again with water to remove remaining traces of chlorophyllin salts. Both washings were added to the small separatory funnel, the contents of which were now washed with 25–50 cc. of ether. The chlorophyllin layer was run into a 100-cc. volumetric flask, made up to the mark with water, and estimated with a colorimeter in comparison with GUTHRIE'S (4) chemical standard.

CHLOROPHYLL DETERMINATION

Quantitative determinations of chlorophyll have been made by different workers but each has used a method of his own. Recently attempts at standardization of the process have been made. SCHERTZ (9) recommends Lovibond color slides as a standard. Only the depth of color was compared here because the slides used did not give the same tint as the chlorophyll solution. That same year GUTHRIE (4) developed a chemical standard to be used in colorimetric determinations of chlorophyll. This standard is a solution which contains 28.5 cc. of copper sulphate solution ($CuSO_4 \cdot 5H_2O$, 10 grams per liter), plus 50 cc. of potassium dichromate solution ($K_2Cr_2O_7$,

20 grams per liter), plus 10 cc. of twice normal ammonium hydroxide, diluted to 100 cc. This solution is colorimetrically equivalent to a solution containing 85 mg. of chlorophyll per liter. GUTHRIE's standard was adopted because it has advantages even over a solution of the pure pigment itself; it is more stable, more easily available, and exact duplications are easily made.

Experimentation

EXPERIMENT I

EFFECT OF SHORT LIGHT PERIOD AND CONTINUOUS LIGHT ON CHLOROPHYLL PRODUCTION

Radishes from which the data were obtained were seeded on three different dates, in both short and continuous light, and harvested at different ages. The Early Carmine Turnips were seeded December 26, 1931; the French Breakfast radishes on March 31 and May 17, 1932. Only one pot of radish plants, a pot of Early Carmine, seeded December 26, 1931, blossomed in the short light period. These blossomed when 146 days old. Of those grown in continuous light the average age for four pots at blooming was 36.8 days. The midrib of the radish leaves was removed before the leaves were used for chlorophyll determinations. At first whole plants were ground up in a Russwin mill and 10-gram portions used for analysis; but this method was soon abandoned for the blades of leaves so that chlorophyll content could be determined on the area basis also.

TABLE I

AGE AND VARIETY OF RADISH PLANTS, AND PERCENTAGE OF CHLOROPHYLL IN THE LEAVES ON GREEN AND DRY WEIGHT BASIS, AND ON AREA BASIS

VARIETY	AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT		
		CHLOROPHYLL GREEN WEIGHT	CHLOROPHYLL DRY WEIGHT	MG/CM².	CHLOROPHYLL GREEN WEIGHT	CHLOROPHYLL DRY WEIGHT	MG/CM².
	days	%	%	*mg.*	%	%	*mg.*
Early Carmine	129	0.117	0.961	.....	.....	.....	.....
Early Carmine	132	0.134	0.690	0.040	.....	.....	.....
Early Carmine	182	0.145	1.320	0.034	.....	.....	.....
French Breakfast	33	0.068	0.861	.....	0.095	0.965	.....
French Breakfast	36	0.095	1.150	0.028	0.104	1.063	0.035
French Breakfast	40	0.107	0.970	0.024	0.097	0.51	0.026

The radish plants in the short light period did not blossom normally; the buds dropped off just before opening. In figure 1, the four plants on the right were grown in the short light period; the two in the center were

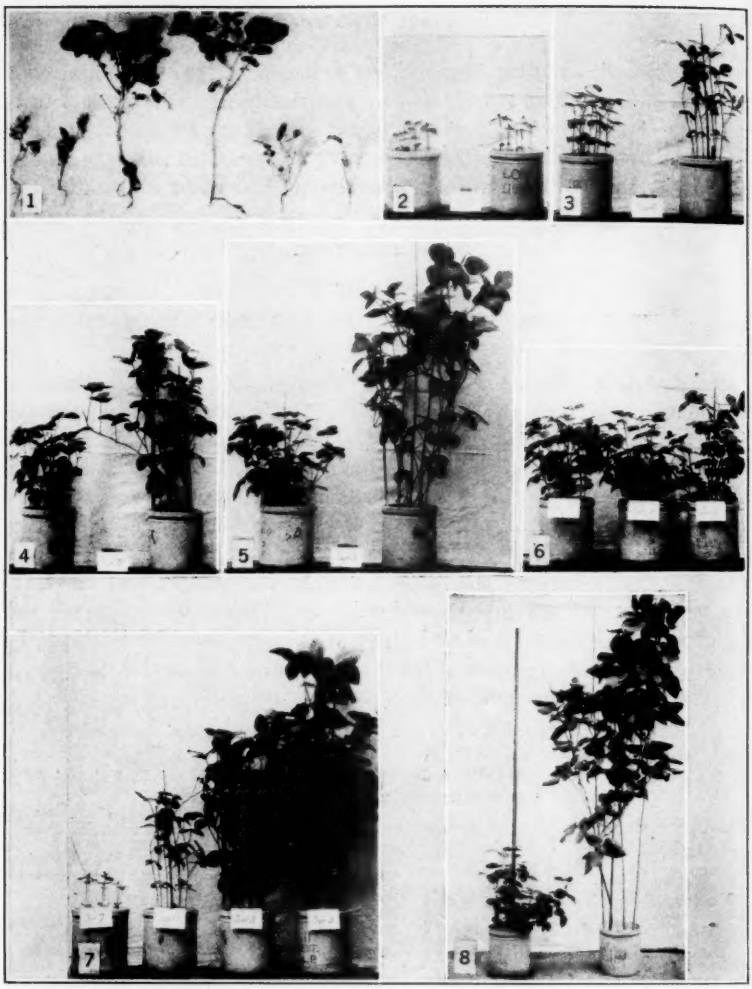

FIG. 1. Radish plants (see text for explanation).

FIG. 2. Soy bean plants 14 days old; short light period plants on left, continuous light plants on right.

FIG. 3. Soy bean plants 30 days old; short light period plants on left, continuous light plants on right.

FIG. 4. Soy bean plants 40 days old; short light period plants on left, continuous light plants on right.

FIG. 5. Soy bean plants 53 days old; same position as in figure 4.

FIG. 6. Soy bean plants all short light period: series 5, 40 days old; series 3, 53 days; series 2, 61 days old.

FIG. 7. Soy bean plants all continuous light plants: series 7, 14 days old; series 6, 30 days; series 5, 40 days; series 3, 53 days old.

FIG. 8. Soy bean plants: short light period plants on left, 80 days old; continuous light plant on right, 109 days old.

TABLE II

AGE OF RADISH PLANTS, PERCENTAGE OF CHLOROPHYLL IN LEAVES ON GREEN WEIGHT, DRY WEIGHT, AND AREA BASIS, AND PERCENTAGE DIFFERENCE IN CHLOROPHYLL CONTENT

AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT			MORE OR LESS THAN IN SHORT PERIOD GREEN WEIGHT	MORE OR LESS THAN IN SHORT PERIOD DRY WEIGHT	MORE OR LESS THAN IN SHORT PERIOD PER CM².
	CHLOROPHYLL GREEN WEIGHT	CHLOROPHYLL DRY WEIGHT	MG/CM².	CHLOROPHYLL GREEN WEIGHT	CHLOROPHYLL DRY WEIGHT	MG/CM².			
days	%	%	*mg.*	%	%	*mg.*	%	%	%
33	0.068	0.861	.....	0.095	0.965	.....	+39.70	+12.07	.....
36	0.095	1.150	0.028	0.104	1.063	0.035	+ 9.47	− 8.18	+25.00
40	0.107	0.970	0.024	0.097	0.510	0.026	−10.30	−47.42	+ 8.33
129	0.117	0.961	.....	.....	.....	.....	.....	.....	.....
132	0.134	0.690	0.040	.....	.....	.....	.....	.....	.....
182	0.145	1.320	0.034	.....	.....	.....	.....	.....	.....

182 days old, and the two on the right were 40 days old. The two on the left were grown in continuous light and are also 40 days old. The four plants on the left are in about the same stage of blossoming, but with a difference of 142 days in age. The two plants in the center, however, grown in the short light period, had reached this stage 36 days before this photograph was taken, and advanced no further; the two on the left, grown

TABLE III

AGE OF RADISH PLANTS AT BLOSSOMING IN SHORT LIGHT AND CONTINUOUS LIGHT PERIODS

VARIETY	SEEDED	SHORT LIGHT PERIOD		CONTINUOUS LIGHT	
		BLOSSOMED	AGE	BLOSSOMED	AGE
			days		*days*
Early Carmine	Dec. 26, 1931	May 20, 1932	146	Feb. 5, 1932	42
French Breakfast	Mar. 4, 1932	.....	.....	Apr. 8, 1932	36
French Breakfast	Mar. 4, 1932	.....	.....	Apr. 13, 1932	41
French Breakfast	Mar. 31, 1932	.....	.....	May 2, 1932	33
French Breakfast	May 17, 1932	.....	.....	June 19, 1932	32
Average age	.....		146		36.8

in continuous light, had just reached this same stage in flowering. The two plants in the center were grown in the same pot in the short light period; one produced a rather large radish while the other showed no enlargement whatever. However, a majority of the radish plants grown in the short period did produce radishes, while in the continuous light enlargement seldom occurred.

The soy beans were planted at irregular intervals in both light periods, and harvested at different ages. Seven plantings were made from March 10 to May 28, 1932, and each planting is called a series; there are seven series. These plants were harvested at different ages, varying in age from 18 to 110 days. A difference can be noticed in the plants in figure 2, seven days after being transplanted and placed in the different light periods. Short period plants are on the left and continuous light plants on the right. These plants were only 14 days old. A marked contrast is noted in figure 3, in which the plants are 30 days old.

The increasing difference with age when grown in short light and continuous light periods is emphasized in figures 4 and 5, 40 and 53 days old respectively. The plants in figure 6 were grown in the shorter period, and are, reading from left to right, 40, 53, and 61 days old. In this light period the plants did not grow beyond a definite size. If vegetative growth is continued it finds expression in numerous shoots from any or all of the nodes.

The opposite condition is found in plants grown in the long light period. Up to the time of harvesting the oldest plant there was apparently an increase in vegetative vigor with age. Figure 7 shows four pots of plants at different ages but all grown in continuous light. Not all the plants in figure 8 are of one age; the seven in the pot on the left grown in the shorter period are 80 days old, and the one plant in the other pot grown in continuous light is 109 days old. New leaves and branches can be seen appearing at the nodes of the plants on the left, grown in the 10-hour light period, while the lower 13 leaves have died and fallen off the plant grown in the 24-hour period.

A marked increase in dry weight per plant in the continuous light period over the other light period was found at all ages. While the percentage increase is consistent it is not in proportion to age. Some of the environmental factors, such as temperature and humidity, that were not controlled may have been responsible for the irregularity. Surely when relative humidity approaches the saturation point with the temperature ranging from 100° to 125° F., the life processes in soy bean plants are not normal.

The ratio between roots and tops was determined for 14 different ages of soy bean plants in both light periods. Seven plants were transplanted when six or seven days old into each of the 2-gallon glazed earthenware pots. One, two, and sometimes three pots of plants were used in each light period to secure sufficient material for the chlorophyll determinations. This accounts for the different numbers of plants indicated in table IV. Table V represents the results from 195 short period plants and from 125 continuous light plants. The ratios in the long light period show a rather sudden jump to 8.50 when 32 days old, with the highest value 9.58 at 37 days. The oldest plant, 110 days old when harvested, had a ratio of 8.82, but this figure may not be reliable. Seven plants had been planted in that pot, but one soon crowded the others so badly that six of them were removed by cutting off at the level of the sand to make room for the large one. When harvested, the roots may not have been separated accurately.

In the shorter period the ratio is more irregular but they also show a wider ratio with increasing age. EATON (3) found the largest root-top ratios in the longest light periods with soy bean plants 38 days old. The same was found to be the case in this experiment up to that age, but with no increase beyond 37 days up to 110 days in continuous light. There was a drop of one-half the ratio in one instance at 42 days. This latter figure might well be disregarded. In the short light period the root-top ratio continues to increase with age and reaches 16.77 for plants 71 days old. EATON'S longest light exposure was only 3.5 hours longer than the short exposure used in this experiment.

TABLE IV

AGE OF SOY BEAN PLANTS, NUMBER, TOTAL DRY WEIGHT, DRY WEIGHT PER PLANT, AND PERCENTAGE INCREASE IN DRY MATTER PER PLANT IN CONTINUOUS LIGHT OVER SHORT LIGHT PERIOD

SERIES	AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT			PERCENTAGE INCREASE PER PLANT OVER SHORT PERIOD
		NUMBER OF PLANTS	TOTAL WEIGHT	WEIGHT PER PLANT	NUMBER OF PLANTS	TOTAL WEIGHT	WEIGHT PER PLANT	
	days		*gm.*	*gm.*		*gm.*	*gm.*	%
6	18	21	4.09	0.194	14	4.43	0.316	62.89
4	25	21	9.29	0.442	14	7.95	0.567	28.28
7	25	21	8.27	0.393	14	10.01	0.715	81.93
2	28	14	4.55	0.325	7	5.41	0.772	137.53
5	28	14	8.34	0.595	14	15.93	1.137	89.11
3	31	9	4.70	0.522	7	12.67	1.810	246.76
5	32	14	10.60	0.757	7	10.91	1.558	105.81
4	34	7	6.10	0.871	7	10.23	1.461	67.78
4	37	7	5.39	0.770	7	20.19	2.884	274.54
6	42	14	19.91	1.422	12	33.48	2.790	96.22
1	55	7	6.70	0.957	7	26.38	3.768	293.73
5	59	18	53.21	2.956	7	66.10	9.442	219.41
3	66	14	48.44	3.460	7	74.95	10.707	209.45
2	71	14	47.85	3.42	....	.....	.....	.....
1	110	.....	.....	.....	1	158.20	158.20	.....

TABLE V

AVERAGE DRY WEIGHT OF TOPS AND ROOTS OF SOY BEAN PLANTS IN SHORT LIGHT AND CONTINUOUS LIGHT PERIODS AND RATIO OF ROOTS TO TOPS

AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT		
	TOPS	ROOTS	RATIO	TOPS	ROOTS	RATIO
	gm.	*gm.*		*gm.*	*gm.*	
18	0.144	0.050	2.88	0.216	0.100	2.16
25	0.251	0.190	1.32	0.444	0.125	3.55
25	0.334	0.059	5.66	0.586	0.128	4.57
28	0.438	0.157	2.78	1.005	0.132	7.61
28	0.271	0.054	5.01	0.678	0.094	7.21
31	0.477	0.444	1.07	1.581	0.228	6.93
32	0.642	0.114	5.63	1.394	0.164	8.50
34	0.685	0.185	3.70	1.261	0.200	6.30
37	0.740	0.107	6.91	2.598	0.271	9.58
42	1.243	0.178	6.98	2.223	0.483	4.60
55	0.857	0.100	8.57	5.490	0.600	9.15
59	2.703	0.252	10.72	8.542	0.900	9.49
66	3.202	0.271	11.81	9.642	1.064	9.06
71	3.225	0.192	16.77	.....	.....	.....
110	.....	.....	.....	142.100	16.100	8.82

The age of the soy bean plants is included in tables IV, V, VI, VII, and VIII so that reference can easily be made from one table to another although not the same number of plants is included in each table. Only dry weight is used in table IV because it was thought that dry weight would be a more nearly accurate representation of the plants as a whole. Little can be said about fresh weight of roots except that some were wetter than others. That being the case the percentage of dry matter in the roots means little or nothing while the dry weight of the roots may be significant.

The largest percentage of increase per plant in continuous light over the shorter period was obtained in the plants harvested on May 4, when 55 days old. These were harvested before the temperature became abnormally high. There were no short light period plants of the same age as the oldest continuous light plant when harvested.

Chlorophyll content was determined on green weight, dry weight, and area basis. There was considerable variation in all three of these, as can be seen in table VI. On the green weight basis the leaves of the plants represented in this table usually show more chlorophyll in the long period than in the short period; 12 groups out of 13 show an increase varying from 5.41 to 53.92 per cent. The leaves showing more chlorophyll in the short light period were from plants harvested on June 19, after a few days of very hot weather, when the thermometer reached 114° F. on several

TABLE VI

AGE OF SOY BEAN PLANTS, CHLOROPHYLL CONTENT OF LEAVES ON GREEN WEIGHT, DRY WEIGHT, AND AREA BASIS, AND PERCENTAGE OF DIFFERENCE IN SHORT AND CONTINUOUS LIGHT PERIOD

AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT			MORE OR LESS THAN IN SHORT PERIOD GREEN WEIGHT	MORE OR LESS THAN IN SHORT PERIOD DRY WEIGHT	MORE OR LESS THAN IN SHORT PERIOD PER CM.²
	CHLOROPHYLL GREEN WEIGHT BASIS	CHLOROPHYLL DRY WEIGHT BASIS	CHLOROPHYLL PER CM.²	CHLOROPHYLL GREEN WEIGHT BASIS	CHLOROPHYLL DRY WEIGHT BASIS	CHLOROPHYLL PER CM.²			
days	%	%	*mg.*	%	%	*mg.*	%	%	%
18	0.195	1.090	0.031	0.281	1.780	0.034	+44.10	+63.30	+ 9.67
25	0.222	1.542	0.035	0.239	1.404	0.032	+ 7.65	− 8.94	− 8.57
25	0.203	1.290	0.024	0.214	1.040	0.028	+ 5.41	−19.37	+16.66
28	0.132	0.965	.....	0.178	1.040	.....	+34.84	+ 7.77	.....
28	0.204	1.641	0.032	0.314	1.848	0.039	+53.92	+12.61	+21.87
31	0.168	1.290	0.023	0.216	1.269	0.032	+28.57	−16.27	+39.13
32	0.225	1.451	0.030	0.263	1.435	0.024	+16.88	− 1.10	−20.00
34	0.208	1.159	0.031	0.256	1.350	0.032	+23.07	+16.47	+ 3.22
37	0.225	1.180	0.037	0.256	1.340	0.035	+13.37	+13.56	− 5.40
42	0.275	1.448	0.035	0.231	1.850	0.035	−16.00	+27.76	− 0.00
55	0.238	1.192	0.034	0.256	1.340	0.037	+ 7.56	+12.41	+ 8.82
59	0.234	1.370	0.037	0.278	1.110	0.032	+18.80	−18.97	+ 1.33
66	0.234	1.064	0.035	0.279	1.111	0.035	+19.23	+ 4.41	− 0.00
71	0.266	1.210	0.040	.....	.....	.....	.....	.....	.....
110	.....	.....	.....	0.230	0.838	0.033	.....	.....	.....

TABLE VII

AGE OF SOY BEAN PLANTS, NUMBER OF LEAVES PER 5-GRAM SAMPLE, SQUARE CM. PER LEAF, AND PERCENTAGE DRY MATTER IN LEAVES GROWN IN SHORT AND CONTINUOUS LIGHT PERIODS

AGE	SHORT LIGHT PERIOD				CONTINUOUS LIGHT			
	LEAVES PER 5-GRAM SAMPLE	AREA PER LEAF	AREA PER GRAM	DRY MATTER IN LEAVES	LEAVES PER 5-GRAM SAMPLE	AREA PER LEAF	AREA PER GRAM	DRY MATTER IN LEAVES
days		*cm.²*	*cm.²*	%		*cm.²*	*cm.²*	%
18	39.00	8.00	62.40	17.85	37.00	11.08	82.00	15.70
25	35.00	9.45	66.20	14.39	26.50	13.98	74.12	17.00
25	38.00	11.10	84.40	15.71	31.50	11.86	74.75	20.60
28	24.00	13.37	64.21	12.43	16.00	25.14	80.45	17.00
31	27.50	13.11	77.29	13.00	15.00	22.11	83.19	17.00
32	24.00	15.37	73.78	15.50	15.00	30.02	90.06	18.50
34	24.50	13.72	67.24	16.66	19.50	20.35	79.40	18.84
37	21.50	14.14	60.84	17.00	10.50	34.38	72.20	19.00
42	15.50	24.82	76.96	19.00	10.75	30.58	65.74	12.50
55	19.50	14.87	58.00	20.00	5.00	68.20	68.20	19.00
59	9.75	31.97	62.35	17.00	6.12	69.54	85.19	25.00
66	13.50	24.54	66.28	22.00	5.06	77.29	78.22	25.00
71	14.00	22.67	65.29	22.00	.....	.....	.....	.....
110	.....	.....	.....	.....	2.85	121.05	69.00	27.50
Average	23.51	16.54	68.09	17.11	15.44	41.19	79.96	19.43

occasions. During such weather the plants were watered three times a day and the floor sprinkled five or six times.

The size of the soy bean leaves varies with the age of the plants. They vary in size from 8 to 31.97 cm.² in the short light period but in continuous light from 11.08 to 121.05 cm.² In other words, the number of leaves per 5-gram sample of fresh weight varies from 39.00 to 9.75 leaves in the former and from 37 to 2.85 leaves in the latter, the larger leaves occurring on the older plants in both light periods (table VII).

In an attempt to determine the efficiency of chlorophyll in the two light periods, the ratio of the chlorophyll in the total leaves of each plant to the total dry weight of each plant respectively was calculated and these differences indicated in table VIII as greater or less than the ratios found for plants of like age in the short light period. The result was not at all striking. The ratios in the shorter period varied from 126 to 284 and in continuous light from 117 to 283. In six of the 13 comparisons the ratio was smaller in the continuous exposure; in the remaining seven the ratio was larger.

TABLE VIII

AGE OF SOY BEAN PLANTS, TOTAL DRY WEIGHT, TOTAL CHLOROPHYLL IN LEAVES, RATIO OF CHLOROPHYLL TO DRY MATTER, AND DIFFERENCE BETWEEN THIS RATIO IN SHORT LIGHT PERIOD AND CONTINUOUS LIGHT

Age	Short light period			Continuous light			Difference in ratio, above or below short light period
	Total dry weight	Total chlorophyll in leaves	Ratio chlorophyll to dry matter	Total dry weight	Total chlorophyll in leaves	Ratio chlorophyll to dry matter	
days	*gm.*	*gm.*		*gm.*	*gm.*		
18	4.09	0.0179	228	4.43	0.0337	131	−97
25	9.29	0.0515	180	7.95	0.0385	206	+26
25	8.27	0.0486	170	10.01	0.0449	223	+53
28	4.55	0.0247	184	5.41	0.0256	211	+27
28	8.34	0.0598	139	15.93	0.1346	118	−21
31	4.70	0.0337	139	12.67	0.0675	187	+48
32	10.60	0.0812	130	10.91	0.0692	157	+27
34	6.10	0.0353	172	10.23	0.0608	168	− 4
37	5.39	0.0423	126	20.19	0.1159	174	+48
42	19.91	0.1354	147	33.48	0.1411	237	+90
55	6.70	0.0358	187	26.38	0.2250	117	−70
59	53.21	0.2270	234	66.10	0.3336	198	−36
66	48.44	0.1960	247	74.95	0.3292	227	−20
71	47.85	0.1684	284	.....	.....	.....	.....
110	.....	.....	.....	158.20	0.5584	283	.....
Average			183			188	+ 5

TABLE IX

AGE OF MANCHU SOY BEAN PLANTS WHEN FIRST PODS APPEARED IN THE SHORT LIGHT PERIOD

Series	Short light period		
	Seeded (1932)	Pod appeared (1932)	Age
			days
1	Mar. 10	Apr. 20	42
2	Apr. 8	May 20	43
2	Apr. 8	May 21	44
3	Apr. 16	May 29	44
5	Apr. 29	June 11	44
Average			43.4

TABLE X

PERCENTAGE OF NITRATE NITROGEN, TOTAL NITROGEN, REDUCING AND NON-REDUCING SUGARS, STARCH, AND TOTAL WATER AND ALCOHOL-SOLUBLE CARBOHYDRATES IN STEMS AND PETIOLES OF SOY BEAN PLANTS

SOY BEAN PLANTS	NO_3 NITROGEN	TOTAL NITROGEN	REDUCING SUGAR	NON-REDUCING SUGAR	STARCH	TOTAL WATER AND ALCOHOL-SOLUBLE CARBOHYDRATES
	%	%	%	%	%	%
Short light period, 25–28 days old	0.37	8.06	1.03	0.04	1.22	2.29
Continuous light, 25–28 days old	0.17	5.55	1.06	0.10	1.40	2.56
Short light, 42 days old	0.32	6.22	1.70	0.46	1.65	3.81
Continuous light, 42 days old	0.13	5.17	2.23	0.38	0.56	3.17

The results shown in table X seem to indicate that nitrate nitrogen was stored in the short light period plants and that the percentage of total nitrogen was also greater in these plants. Carbohydrates may have been the limiting factor in the shorter period which was caused by the limited exposure to light.

EXPERIMENT II

EFFECT OF IRON AND MANGANESE ON CHLOROPHYLL PRODUCTION IN YOUNG CORN PLANTS

Zea mays saccharata, the garden variety Golden Bantam, was used in this experiment. The plants on which chlorophyll determinations were made were harvested June 16, when 34 days old. One pot in each treatment was harvested. Sixteen days later, when it appeared that the plants were becoming crowded, two more pots in each treatment were harvested; only fresh and dry weight determinations were made on these. The gain in green weight and dry weight on the addition of both Fe and Mn, or of Fe or Mn added separately, compared with the condition when both were absent, can be considered.

Four separate treatments were used: the plants in treatment A received neither iron nor manganese; those in B received 1 p.p.m. of Fe; those in C received 1 p.p.m. of both Fe and Mn; and those in D received 15 p.p.m. Fe and 1 p.p.m. Mn in the nutrient solution applied by the drip culture method.

TABLE XI

CHLOROPHYLL PRODUCTION IN YOUNG CORN PLANTS WITH PARTS PER MILLION OF IRON AND MANGANESE IN THE NUTRIENT SOLUTION

	A	B	C	D
P.P.M. Fe	0	1	1	15
P.P.M. Mn	0	0	1	1
Weight of chlorophyll in 5 grams of fresh leaves	*gm.* 0.0077	*gm.* 0.0095	*gm.* 0.0100	*gm.* 0.0127
Percentage of chlorophyll in leaves	% 0.1530	% 0.1906	% 0.2000	% 0.2612
Percentage increase in chlorophyll over A	.....	24.5751	30.7189	70.7189
Percentage increase in chlorophyll over B	.....	.....	4.9317	37.0409
Percentage increase in chlorophyll over C	.....	.....	.....	30.6000
Cm.^{2} per 5-gram sample of fresh leaves	*cm.*^{2} 352.1000	*cm.*^{2} 371.2000	*cm.*^{2} 388.2000	*cm.*^{2} 415.8000
Chlorophyll, mg./cm.^{2}	0.0218	0.0255	0.0257	0.0305
Percentage of dry matter in leaves	% 11.5000	% 12.0000	% 14.0000	% 14.5000

An experiment on nitrogen assimilation by sugar-cane and sweet corn plants at different soil temperatures (**6**) was being carried on at the same time, and just prior to harvesting the sugar-cane plants, chlorophyll determinations were made on some of the leaf-blade material. These sugar-cane plants were grown in the same type of quartz sand as was used for the soy bean, radish, and corn plants. But the pots were placed in automatically controlled, electrically heated water tanks to provide soil temperatures of 15°, 25°, and 30° C. Tanks 1 and 2 were to be kept at 15° but for a time during August this temperature was exceeded. Tanks 3 and 4 were kept at a temperature of 25° throughout the experiment, and tanks 5 and 6 at 30° C. The plants in tanks 1, 3, and 5 received their nitrogen as $(NH_4)_2SO_4$ and NH_4OH, while those in tanks 2, 4, and 6 received their nitrogen as $Ca(NO_3)_2$. Both nutrient solutions had a reaction value of pH 7.

The leaves used for chlorophyll analyses were in every instance the third from the top of the plants.

TABLE XII

AVERAGE FRESH WEIGHT PER CORN PLANT UNDER DIFFERENT TREATMENTS WITH Fe AND Mn, GAIN IN WEIGHT PER PLANT AND PERCENTAGE OF GAIN OVER PLANTS NOT RECEIVING Fe AND Mn

	A		B		C		D	
P.P.M. Fe	0		1		1		15	
P.P.M. Mn	0		0		1		1	
	NUMBER OF PLANTS	GREEN WEIGHT	NUMBER OF PLANTS	GREEN WEIGHT	NUMBER OF PLANTS	GREEN WEIGHT	NUMBER OF PLANTS	GREEN WEIGHT
		gm.		*gm.*		*gm.*		*gm.*
34 days old	14	10.90	16	15.43	17	22.42	13	23.19
				%		%		%
Percentage gain over A	.....	.....	.....	41.55	.....	105.68	.....	112.75
		gm.		*gm.*		*gm.*		*gm.*
50 days old	31	39.49	27	55.76	29	54.71	28	58.49
				%		%		%
Percentage gain over A	.....	.....	.....	41.20	.....	38.54	.....	48.11

TABLE XIII

DRY WEIGHT PER CORN PLANT UNDER DIFFERENT TREATMENTS WITH Fe AND Mn

	A		B		C		D	
P.P.M. Fe	0		1		1		15	
P.P.M. Mn	0		0		1		1	
AGE	NUMBER OF PLANTS	DRY WEIGHT	NUMBER OF PLANTS	DRY WEIGHT	NUMBER OF PLANTS	DRY WEIGHT	NUMBER OF PLANTS	DRY WEIGHT
		gm.		*gm.*		*gm.*		*gm.*
34	14	1.75	16	1.05	17	1.54	13	1.75
50	31	3.27	27	4.85	29	4.80	28	5.71
Gain in weight in 16 days	.....	2.52	.....	3.80	.....	3.26	.....	3.96
				%		%		%
Percentage gain in 16 days over A	.....	.....	.....	50.79	.....	29.36	.....	57.14

TABLE XIV

PERCENTAGE OF CHLOROPHYLL IN SUGAR-CANE LEAVES ON GREEN AND DRY WEIGHT BASIS, CHLOROPHYLL PER SQUARE CM., AND PERCENTAGE OF DRY MATTER (6)

TANK	PERCENTAGE OF CHLOROPHYLL		MG./CM.^{2}	DRY MATTER	PROPORTION OF CHLOROPHYLL	
	GREEN WEIGHT	DRY WEIGHT			GREEN WEIGHT BASIS	AREA BASIS
	%	%	*mg.*	%	%	%
1	0.220	0.903	0.0389	24.4	100.00	100.00
2	0.263	1.196	0.0438	22.0	119.52	112.59
3	0.223	1.016	0.0420	22.0	101.36	107.96
4	0.252	1.031	0.0500	24.4	114.54	128.53
5	0.202	0.886	0.0379	22.8	91.81	97.42
6	0.204	0.825	0.372	24.8	92.72	95.63

Discussion

The amount of chlorophyll contained in leaves necessarily limits the amount of photosynthesis under conditions as they normally exist in nature. As early as 1879, WEBER (MILLER 7) noticed that equal areas of leaves of different plants grown under similar conditions had different assimilatory powers. In 1882 HABERLANDT attempted to explain this by counting the chloroplasts in a unit area, but MILLER concludes that since there is no evidence that all chloroplasts contain the same amounts of chlorophyll no definite conclusions can be drawn from this method.

It was found by LUBIMENKO (5) that shade plants contained more chlorophyll than sun plants, and could accomplish the same amount of photosynthesis with less illumination than sun plants. He noted that if plants are grown under lowland and alpine conditions, the lowland plants may have as much as 2.3 times more chlorophyll than the alpine plants of the same species. Here there is a marked difference in the light as well as in other growth conditions. If light were the only variable factor in the environment, what differences in chlorophyll production would be found in the same species of plants?

Soy bean and radish plants were grown with this question in mind. The method adopted was to grow one set of plants in daylight from 8 A.M. to 6 P.M., supplemented by artificial light on cloudy days, and the other set in the total natural daylight supplemented as described, plus artificial light the remaining hours of the 24. During the winter season, both sets of plants received the same amount of daylight, but as the days became longer

the continuous light plants received more and more daylight in proportion to the amount of artificial light received. It would seem that this condition might be favorable to the continuous light plants.

The soy bean, a so-called short-day plant, and the radish, a long-day plant, were chosen because they were contrasting plants so far as light requirements for seed production were concerned. Under continuous light, radish plants produced flowers in from 32 to 42 days, with an average of 36.8 days. Only one pot of radishes was grown long enough in the short light period for flower production. These were planted December 26, 1931, and blossomed May 20, 1932, 146 days later. The radishes planted on the same date in continuous light blossomed February 5, 1932, when 42 days old. Temperature or light may be a factor here.

A negative correlation seems to exist between temperature and the age of radish plants at blossoming. Those seeded December 26, 1931, and grown in continuous light blossomed in 42 days, while those seeded May 17, 1932, and grown in continuous light blossomed in 32 days, a difference of more than 31 per cent. of the time required in late spring. Growth form varied considerably in both light periods, especially in the short light period. This may be noticed in figure 1.

Length of exposure to light seemed to have the opposite effect on soy bean plants. In no case were opened flowers seen on the plants, so the age used here is that at which pods were first seen in the short exposure. The range was very narrow, 41 to 44 days as compared with 32 to 42 days for radishes in continuous exposure. No pods occurred on any soy bean plant grown in continuous light although one plant was harvested when 110 days old.

The vegetative vigor of soy bean plants seemed to increase with age in continuous light. The leaves became enormous in size; only 2.85 leaves were required for 5-gram samples of fresh leaves for chlorophyll analysis. These averaged 121.05 cm.^{2} per leaf. Bean plants of series 3 were harvested in both light periods when 66 days old, and 13.50 leaves with an average area of 24.54 cm.^{2} were required for 5-gram samples of fresh leaf material in the short exposure, while only 5.06 leaves with an average area of 77.29 cm.^{2} were required in the continuous exposure. The leaves produced in continuous light have a surface area more than three times as great as those produced in the short exposure. At 32 and 18 days the figures are 24 leaves with areas of 15.37 cm.^{2}, 15 leaves with areas of 30.02 cm.^{2}, and 39 and 37 leaves with areas of 8.00 and 11.08 cm.^{2} respectively (table VII).

The thickness of the soy bean leaves was not determined, but the fourth and eighth columns of table VII indicate an appreciable difference. One gram of the fresh leaf blade material in the continuous light for 13 dupli-

cate measurements of 5 grams each averages 79.96 cm.^{2}, and in the short exposure only 68.09 cm.^{2} This average difference of 11.87 cm.^{2} of area per gram shows that the leaves were thinner in the continuous light. The leaves in the short exposure felt more leathery to the touch than the others, but the greater length and also possibly a greater number of leaf hairs in the long exposure may have accounted for that.

Only the leaves were analyzed for chlorophyll content. At an early age the plants in continuous light would naturally be expected to contain more chlorophyll than those of a corresponding age in the shorter light exposure. However, two analyses were made of 25-day old plants that had more chlorophyll in the short light period. There is considerable variation in the total chlorophyll content of the leaves as can be seen from the data of table VIII. On the green weight basis, for 13 duplicate determinations in both light periods, the average chlorophyll content was 19.83 per cent. more in continuous light; on the dry weight basis 7.20 per cent. more; and on the area basis 6.67 per cent. more than in the short exposure. Radish leaves showed an average of 12.95 per cent. more chlorophyll in continuous light on the green weight basis; 14.53 per cent. less on dry weight basis; but 16.66 per cent. more on the area basis (calculated from data of tables I and II).

An attempt was made to discover whether there is any correlation between the total dry weight and the chlorophyll content of the leaves of soy bean plants by dividing the dry weight by the amount of chlorophyll in the total leaves, assuming the remaining leaves contained the same amount of chlorophyll as the 5-gram samples used in analysis (table VIII). A high ratio would indicate greater efficiency than a lower ratio, other things being equal in the two periods; but things were not equal. There are many possibilities for errors. The greatest single error might easily be due to the fact that in the continuous exposure many of the lower leaves dropped off before analyses were made; consequently an indeterminable amount of chlorophyll was lost. No leaves dropped off the plants in short exposure, so that a more nearly accurate value for chlorophyll was obtained.

In the plants analyzed for chlorophyll when 18 days old, corresponding leaves (*i.e.*, the first two foliage leaves and one compound leaf) were gathered from each plant. Leaves from 14 plants were used in the continuous light experiments and from 21 plants in the 10-hour day experiments. The ratios of chlorophyll to dry matter in this instance are 1:228 and 1:131 in the short and continuous exposures respectively. There is an increase of chlorophyll in the total leaves in continuous light of 87.87 per cent., and the 14 plants in this set produced 8.31 per cent. more dry weight than did the other 21. But when this increase is placed on a "per plant" basis, the chlorophyll increase is 182 per cent., and the dry weight increase (table IV)

is 62.89 per cent. per plant. In view of the fact that growth conditions in each light period were maintained as nearly identical as possible, these irregularities are striking.

Cross-sections of soy bean leaves were examined under a microscope and it was thought a difference in arrangement of the chloroplasts could be noticed. The chloroplasts were more or less plano-convex, and in the continuous light these were arranged more regularly, with their flat sides toward the cell wall.

In experiment II with corn a different approach to the problem of chlorophyll production was attempted. It is conceded that chlorophyll is not formed in the absence of iron and that only very small quantities are required. The exact function of iron in chlorophyll production is not known since iron does not occur in the chlorophyll molecule. MILLER (7) cites ODDO and POLLACCI as stating that *Zea mays, Solanum nigrum, Datura stramonium, Euphorbia* sp., and *Aster sinensis* were grown in a nutrient solution in which Fe was replaced by the magnesium salt of pyrrole-carbonic acid. These investigators think that Fe has a catalytic action on the formation of the pyrrole nucleus which is the center of the chlorophyll complex. If the pyrrole nucleus is already present they think that Fe is not necessary for the formation of chlorophyll.

WILLSTÄTTER'S observations seem to support this theory, but DEUBER (2) could not confirm it. He grew corn, cow pea, soy bean, and *Spirodela* in nutrient solutions containing different proportions of a magnesium salt of pyrrole-carbonic acid substituted for iron, but in no case did this compound prevent chlorosis of the leaves of the plants.

Corn plants were grown in no. 3 quartz sand of the following composition: SiO_2, 98.95 per cent.; Al_2O_3, 0.6 per cent.; iron as Fe_2O_3, 0.31 per cent.; and no calcium nor magnesium. A nutrient solution made up of chemically pure salts was used with this sand in four different treatments as previously described. In A, the culture not receiving iron in the nutrient solution, 5-gram duplicate samples of fresh leaves were found to contain 0.1530 per cent. chlorophyll (table XI). In B, with the addition of Fe 1 p.p.m., a chlorophyll content of 0.1906 was found, a 24.75 per cent. increase over A. With the addition of Fe and Mn each 1 p.p.m., a very small increase in chlorophyll content was obtained in C; but when Fe 15 p.p.m. and Mn 1 p.p.m. were added to culture D a 30.6 per cent. increase over C was obtained. This makes a total increase of 70.71 per cent. over culture A.

The effect of Mn 1 p.p.m. with no Fe, or Mn 15 p.p.m. and Fe 1 p.p.m. in contrast to cultures B and D, was not determined; but the data indicate that iron was more effective, under the conditions of this experiment, than manganese in stimulation of chlorophyll production.

In experiment II there is a gradual increase in dry weight with the increase in chlorophyll content. The thickness of the leaves decreases with the increase in chlorophyll content per cm.^{2} The increase in dry matter, the increase in chlorophyll content on the green weight basis, and the increase in the number of cm.^{2} per gram of fresh leaf material are positively correlated in this experiment.

An increase in chlorophyll production in corn up to 67 per cent. over the check was obtained by SHULL and MITCHELL (**10**) with the use of x-ray treatments. The seeds were treated for 1, 2, 3, 4, and 5 minutes through a 1-mm. aluminum filter. The greatest increase occurred in the 2-minute treatment but all treatments showed an increase over the control.

In the experiment on the assimilation of nitrogen as NO_3 or as NH_3 by sugar-cane at soil temperatures of 15,° 25°, and 30° C., a marked difference in chlorophyll content was observed. Considering the chlorophyll content of the plants receiving nitrogen as NH_3 in the 15° C. tank as 100 per cent., the most chlorophyll was found in the NO_3 treatment at 25° C. soil temperature on the area basis. At all three soil temperatures more chlorophyll was found in the nitrate nitrogen treated plants than in those receiving their nitrogen as ammonium nitrogen. The highest percentage of chlorophyll on both the green weight and the dry weight basis occurred in the NO_3 treated plants at a soil temperature of 15° C. (table XIV).

Summary

1. Radish plants continued vegetative growth in a 10-hour day for 182 days and were still vigorous when harvested. Those in continuous light blossomed in about 36 days, soon ripened seed, and died. Radish plants in the short light period tended to store food while those in continuous light seldom produced enlarged storage organs.

2. Size of leaves in the soy bean increased with age in both light periods, but much more rapidly in continuous light. When 66 days old the leaves in continuous light were more than three times as large as those of the same age in the 10-hour period.

3. The leaves tend to be thinner with continuous light exposure, although this difference may not be significant.

4. On the average, leaves developed in continuous light contain more chlorophyll on the green weight, dry weight, and area basis than do those developed in a short light period.

5. Vegetative activity in soy beans seemed to increase with age in continuous light exposure; the reverse was the case with a short exposure.

6. Chlorophyll production was markedly stimulated in corn plants by the addition of Fe 1 p.p.m. and 15 p.p.m. to the nutrient solution.

7. Little response was obtained with Mn 1 p.p.m. Impurities in the sand may have been the limiting factor.

8. The percentage of dry matter of corn leaves increased in inverse proportion to the thickness of the leaves but in direct proportion to the chlorophyll content on green weight, dry weight, and area basis.

9. Gains in chlorophyll content are reported in corn plants as a result of x-ray treatments.

10. Under conditions of a nitrogen assimilation experiment with sugarcane, more chlorophyll was produced by the plants receiving nitrogen as NO_3 than by those receiving nitrogen as NH_3 in each of three soil temperatures.

Appreciation is hereby expressed to Drs. E. J. KRAUS and C. A. SHULL for their assistance, suggestions, and criticisms given throughout this work.

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RESPONSES OF KENTUCKY BLUEGRASS TO VARIATIONS IN TEMPERATURE, LIGHT, CUTTING, AND FERTILIZING[1]

CARTER M. HARRISON

(WITH SEVEN FIGURES)

Of primary importance among the several environmental factors which influence the production and maintenance of turf are seasonal variations in temperature, amount and intensity of sunlight, and cutting and fertilizing practices. It has long been observed that bluegrass grows best during the cooler seasons, although the reasons for such behavior have never been fully understood.

When bluegrass is cut short and heavily watered or fertilized, especially during the hot summer months, undesirable results follow. The turf thins out, the production of new leaves ceases, and during the cooler, wetter period of fall large numbers of the plants fail to recover. In attempts to maintain a vigorous green growth, nitrogenous fertilizers are often added to such turf when actually such an addition may be harmful instead of beneficial, especially during the hot weather of summer.

With a view to determining a possible cause for the widely differing results often obtained from apparently similar practice, the following experiments were conducted in sand culture. All cultures were obtained from the vegetative propagation of one original bluegrass plant. More particularly the experiments dealt with: (a) the effect of cutting to 0.5 inch, 1 inch, and 2 inches, plants which were supplied either with a solution high in ammonium nitrogen and low in nitrate nitrogen, or one high in nitrate and low in ammonium nitrogen; (b) the type of growth produced at different seasons of the year and the effect upon such growth of varying degrees and times of defoliation; (c) the effect of cutting plants grown with a continuous nitrogen supply as contrasted with some which had had no nitrogen during a period beginning 6 weeks prior to the initial cutting and extending to the close of the experiment; (d) the type of recovery growth produced when cultures were cut back to 1 inch in height every ten days, at 60°, 80°, and 100° F., with and without a nitrogenous fertilizer. The experiments were conducted in the University of Chicago greenhouse, from the fall of 1930 to the fall of 1932.

Several recent papers contain literature reviews of some length on much the same subjects as taken up here. GRABER, NELSON, LUEKEL, and ALBERT (**2**), PIERRE and BERTRAM (**7**), LUEKEL and COLEMAN (**6**), and GRABER (**1**) have published recent papers on the food reserves of grasses in relation to

[1] Contribution from the Hull Botanical Laboratory, the University of Chicago, under a fellowship granted by the United States Golf Association, Green Section.

growth. HARRISON (3) has published a recent paper on the effect of cutting on grass development. KRAUS and KRAYBILL (5) and THOMAS (9) have fully covered the present literature on carbohydrate-nitrogen relationships.

Experimental data

EXPERIMENT I

EFFECTS OF CONTINUED CUTTING AND FERTILIZING.—An individual Kentucky bluegrass plant (*Poa pratensis*) was propagated vegetatively, split up into 4-gm. segments and planted one segment each in 2-gallon glazed pots containing white quartz sand, free of fertilizers. The plants were started April 1, 1931, and supplied with a four-salt nutrient solution, selected from the ammonium sulphate series of JONES and SHIVE (4) made up of calcium nitrate, potassium phosphate, magnesium sulphate, and ammonium sulphate in distilled water. This was supplied by the constant drip method similar to that used by ROBBINS (8), one half of the cultures receiving a solution high in nitrate and low in ammonium nitrogen ($T_3R_1C_5$) and the other half receiving a solution high in ammonium and low in nitrate nitrogen ($T_3R_5C_1$). The pH of the solution high in nitrate was approximately 4.5, while that of the solution high in ammonium was 4.8. The cultures were grown without disturbance until September 22, when five pots of each set (ammonium high and nitrate high) were taken down and the sand washed from the roots. Figure 1 gives an idea of the different types of growth of

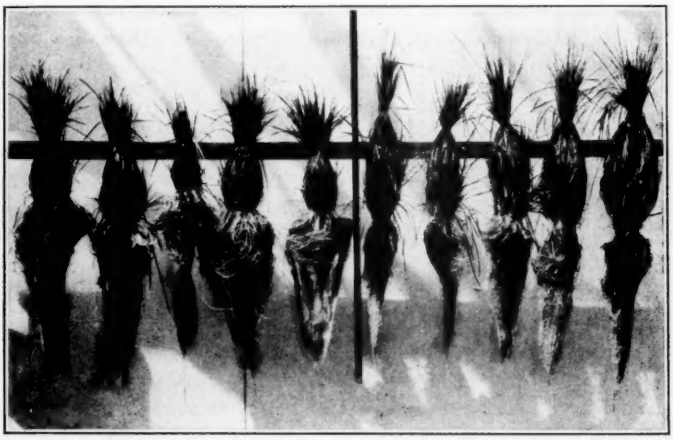


FIG. 1. Cultures on the left were grown in the solution high in nitrate and low in ammonium nitrogen, while those on the right were grown in the solution high in ammonium nitrogen and low in nitrate nitrogen. Note the habit of growth and the far greater number of rhizomes at the left.

the two sets. Table I gives the weights of the plant parts in grams after drying.

TABLE I

DRY WEIGHT OF PLANTS SUPPLIED WITH HIGH NITRATE AND HIGH AMMONIUM SOLUTION

TYPE OF SOLUTION		CULTURE NUMBER					
		1	2	3	4	5	Av.
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
High nitrate-low ammonium	Roots	9.5	9.1	9.5	8.5	5.2	8.3
	Tops	44.5	41.2	39.0	32.5	23.5	36.1
	Rhizomes	4.3	4.1	5.7	5.0	4.0	4.6
High ammonium-low nitrate	Roots	7.5	7.3	6.5	3.7	3.5	6.3
	Tops	42.5	38.0	30.5	19.0	16.5	29.3
	Rhizomes	2.5	2.2	1.8	0.7	1.0	1.6

The plants receiving the solution high in ammonium and low in nitrate nitrogen had leaves 15 to 22 inches long, which lay flat or drooped over the sides of the pots and were very dark green; those supplied with the solution high in nitrate and low in ammonium nitrogen had leaves 8 to 12 inches long, which were lighter green in color and stood upright in the pots. There were from 300 to 500 rhizomes in the cultures supplied with the high nitrate solution, as compared with 50 to 150 in the cultures supplied with the high ammonium solution. The former were short and thick and considerably branched as compared with the latter, which were long, thin, and unbranched.

On the same date (September 22) the leaves of five cultures of each series (high NH_4 and high NO_3) were cut to 0.5, 1, and 2 inches respectively. The clippings from this initial cutting were dried and weighed. One week later the grass was again clipped back to the same heights. It was noticed that the shorter the cutting height the shorter was the new growth made after cutting. In both sets the grass cut to 0.5 inch grew approximately 4 inches, that cut to 1 inch grew 5 inches, and that cut to 2 inches grew 6 inches. The measurements were taken above the point of the original cutting in each case. After the second cutting, during a period of high greenhouse temperatures, it was observed that the grass recovered more slowly than usual. The new leaf growth would extend upward from 1 to 3 inches and then die back from the tips of the blades. It was much more noticeable in the plants supplied with ammonium nitrogen than in those supplied with the high nitrate solution. A considerable number of the above-ground buds soon failed to produce additional new leaves or to

extend those which had been cut, and upon examination they were found to be dead. Most of the plants which died soonest were in the center of the culture, which was the oldest portion. The plants started growth from the segment which was the original planting and new rhizomes extended laterally from this segment. These new rhizomes were younger than the stem from which they originated, and none of their potential leaves had extended upward. Consequently when cutting was begun, a larger proportion of the leaf area of the older portion of the plant was removed than was true in the case of the rhizomes which had just pushed above the surface of the soil or were as yet buried beneath it. The number of leaves that a given bud will produce is fixed at an early stage in its growth. The leaves in the older buds had practically all unfolded and matured, whereas in the younger rhizomes the leaves had either just begun to unfold from the bud or as yet remained underground.

The underground parts were examined to determine what was taking place under the conditions of frequent cutting, high fertilization, and high temperatures. The cultures supplied with nitrate had from 300 to 500 growing rhizome tips on September 22 before cutting was begun, and the ones supplied ammonium nitrogen had from 50 to 150. These were all below the soil line, extending vegetatively, and producing scale leaves. On examination October 15, after the two subsequent cuttings in September, it was found that every rhizome tip below the soil line, in either fertilized set and at all three cutting heights, was dead. The terminal buds of the underground rhizomes were brown and beginning to decay. The death of the tissue back from the tip depended somewhat upon the amount of green leaf tissue remaining after cutting; the more of the green tissue removed, the greater the degree of killing of the rhizome when measured back from the terminal point. The terminal bud died first and then successively the buds at the nodes back from the tip. In most cases the entire rhizome became brown and decayed. The roots on these rhizomes died and in some cultures as much as 90 per cent. of the portion above ground was killed. The younger stems toward the periphery of the culture survived. Any rhizome that had recently emerged above the soil line and was actively producing vegetative leaves also survived. As soon as these rhizomes became above-ground stems, they produced green vegetative leaves only. These new leaves were green even below the point of shortest cutting, and probably they manufactured sufficient carbohydrates to maintain the new rhizome. The older stems had a considerable number of dry leaf sheaths at their bases, however, each new leaf produced having to extend through these dead leaves before emerging into the light. Consequently at each cutting all of the green leaf tissue produced during the period since the previous cutting was removed. Figure 2 shows how the check plants and

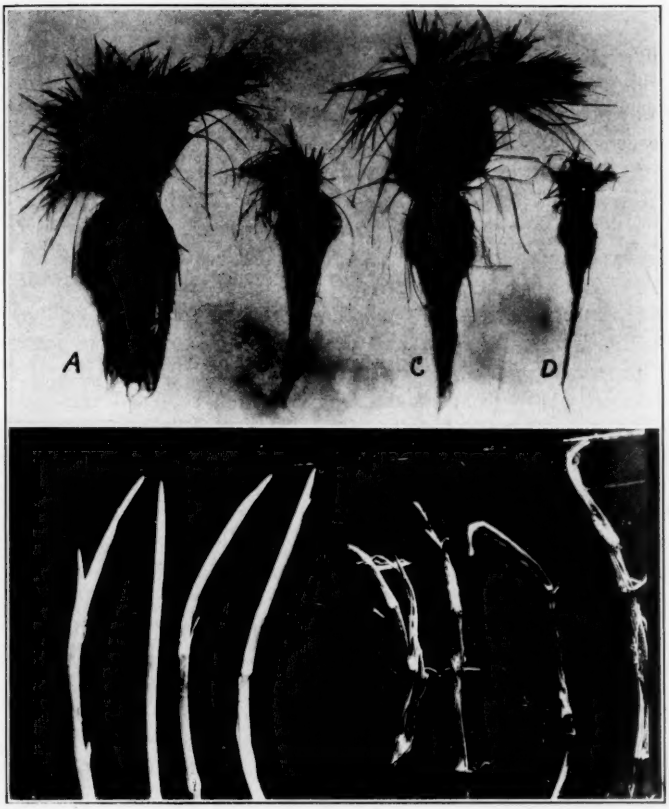


FIG. 2. Above: effects of cutting treatments on bluegrass plants. Plants *A* and *C* are uncut controls; plants *B* and *D* were cut down to 0.5 inch twice, the cuttings one week apart. The new growth apparent on *B* and *D* is that which has appeared since the last cutting 20 days previous. All the cultures were grown in sand and supplied with a nutrient solution: *A* and *B* received a solution high in nitrate and low in ammonium nitrogen, while *C* and *D* received a solution high in ammonium and low in nitrate nitrogen.

Below: at the left, rhizomes from an uncut control; at the right, rhizomes from plants cut back to 0.5 inch in height twice, the cuttings one week apart. Photographed 20 days after second cutting.

some that were cut twice appeared on October 20, approximately one month after the initial cutting. The rhizomes are enlarged about two times in figure 2. It will be noted that the rhizomes from the culture not cut are white and plump with sharp terminal points, whereas those taken from the culture that had been clipped short twice are dark and shriveled.

Experiment II

Growth of bluegrass plants at different seasons.—The bluegrass plant, when started from a vegetative segment without rhizomes, began new growth by tiller-like buds in the axils of the leaves. These buds were green on emergence and grew upright almost from the start. They produced a few green scale leaves at the base but most of the leaves were large. After this initial start, short stolons which grew 1 or 2 inches from the crown of the plant before they turned upward were produced. These produced more scale leaves than the first tiller-like buds, and roots arose near the nodes. Later short rhizomes were produced. These gave rise to scale leaves underground, but upon emergence from the sand, a short way from the crown, developed vegetative leaves. This process continued until the pot was full of upright stems. Then, when the days were long and bright, the active production of above-ground stems was somewhat retarded and there were produced below the soil line large numbers of rhizomes which did not emerge so long as the top of the plant was not cut back, or so long as the days remained long and bright.

These rhizomes produced scale leaves only, and grew in length, often around the inside of the pot and sometimes down to the bottom. In late fall and early winter, as the sunlight diminished with day length and through interspersed dark cloudy periods, few new rhizomes were produced by grass that was continually supplied with nitrogenous salts. The rhizomes which were produced below the soil level during the summer and early fall, and which produced scale leaves only, gradually turned upward at the growing point, and vegetative leaves instead of additional scale leaves were produced; the new leaves turned yellowish green and emerged above the soil line. The development and emergence of these rhizomes continued until there were no growing tips below the soil line. On plants which were heavily fertilized with nitrogenous materials, during the dark winter period, a considerable number of these rhizomes as well as roots died, probably because of a deficit of carbohydrates, either as stored substance or because of the inability of the plant to manufacture them in quantity during the short cloudy days. During this period no new roots nor rhizomes were produced to take the place of those which died.

Following the emergence of the underground rhizomes, growth was initiated in the buds in the axils of the leaves above ground, and many short tiller-like branches were produced, much the same as when the plants started growth from the original plant segment. In other words, the 300 to 500 rhizomes that were produced below the soil line during summer and early fall, when the days were long and sunny, gradually emerged as the amount and intensity of light decreased, and the production of scale leaves

by these rhizomes ceased and vegetative leaves were produced. On the other hand, plants that were supplied with a minus-nitrogen nutrient solution after November 10 had produced a considerable number of new rhizomes by February 1. These increased somewhat in diameter and remained below the soil line. The tops of these plants, when the days were short and cloudy, turned yellow and grew little if any, while the root system grew deeper and more extensive. It appeared that nitrogen added at a time when the days were short and cloudy was necessary to cause emergence above the surface of the soil of rhizomes which were produced during a period of long sunny days; and that the cultures which received no nitrogen, but which were exposed to the same short cloudy days, actually continued to develop their root systems and to produce new rhizomes, with a cessation of active extension of the top. There was some death of rhizomes and roots in the cultures which were supplied continuously with nitrogenous materials. The plants supplied the minus-nitrogen solution appeared to be at a standstill both below as well as above ground, after about eight months of the treatment; and as the days became long and bright nitrogen probably became the limiting factor in growth. On the other hand, lack of sufficient light for carbohydrate synthesis in plants heavily fertilized with nitrogen probably was the limiting factor during the winter months. The difference in growth response exhibited by plants grown with and without nitrogen during the winter months is of considerable interest. The cutting factor was then introduced to see what added effect it would have on the behavior of the plants. It was found that the cutting of a plant grown under winter light conditions and supplied with nitrogen was much more disastrous than cutting one that had had no nitrogen but which had had additional light artificially added for 14 hours per day after November 10. The plants were cut back to approximately 1 inch. Table II shows how the production of top growth compared.

TABLE II

WEIGHTS OF THE CLIPPINGS PRODUCED BY TWO DIFFERENT CULTURES, ONE HAVING ADDED NITROGEN AND GREENHOUSE LIGHT ONLY, AND THE OTHER HAVING ADDED ARTIFICIAL LIGHT BUT NO ADDED NITROGEN

CULTURAL CONDITIONS	DECEMBER 15		DECEMBER 23	JANUARY 6
	INITIAL CUT GREEN WEIGHT	DRY WEIGHT	DRY WEIGHT	DRY WEIGHT
	gm.	*gm.*	*gm.*	*gm.*
−N, + added artificial light	173	46.7	2.8	1.4
+N, greenhouse light only	176	42.0	1.3	0.3

The culture grown with nitrogen and greenhouse light only was about 90 per cent. dead at the end of the three cuttings. When cutting was begun it had only a very few slender rhizomes. Of these, first the tips and finally the whole rhizomes died. When tips were cut from these rhizomes none of the buds near the nodes back from the cut end started growth, and in most cases the entire rhizome died. A considerable number of roots also died. Ten days after the last of the three cuttings, when the plant was lifted from the pot, only 3 or 4 inches of sand adhered to the roots. At the beginning of the test, all the sand in the 10-inch pots came out as a compact mass, held together by many fine roots.

In contrast to the culture receiving added nitrogen and natural light only, that grown without nitrogen and with added artificial light was growing vigorously after the last of three cuttings. The new growth produced, after cutting off the old yellow leaves, was dark green and became more succulent with each cutting. The large number of stocky rhizomes below the soil line, present when cutting was begun, stopped producing scale leaves and grew vegetative leaves which turned upward and finally emerged above the soil line. When tips were cut from the rhizomes in this culture, buds near the nodes back from the cut end started to grow. The first bud from the cut end elongated about 0.5 inch before dying; the second, about 1 inch; but the third one elongated sufficiently to emerge above the soil line, produce vegetative leaves, and survive. At the end of the test the roots permeated the soil mass of the entire pot, making it possible to empty the pot by lifting the plant out.

As a result of giving artificial light during the winter and withholding the supply of nitrogen, the plants if left uncut turned yellow, the leaves became stiff and upright, and very few new stems were produced above ground while a considerable number were produced as rhizomes below the soil line. When given added artificial light in the winter and a continuous nitrogen supply, the plants remained succulent and green, the axillary buds elongated above ground, produced green leaves, and there was very little if any production of underground stems. As soon as the days became longer and brighter, in March and April, the plants supplied with nitrogen produced not only a greater amount of top growth than those receiving no nitrogen but also more new roots and rhizomes.

Plants that were left to grow for a period without cutting with a continuous nitrogen supply and then cut back short appeared to undergo a reduction in the amount of underground parts, namely, the roots and rhizomes. There seemed to be a balancing of the underground portion in relation to the amount of top remaining. Then, if cutting was suspended, these plants began to produce new top growth, rhizomes, and roots much sooner than a plant that was left uncut; and in two months of long, sunny

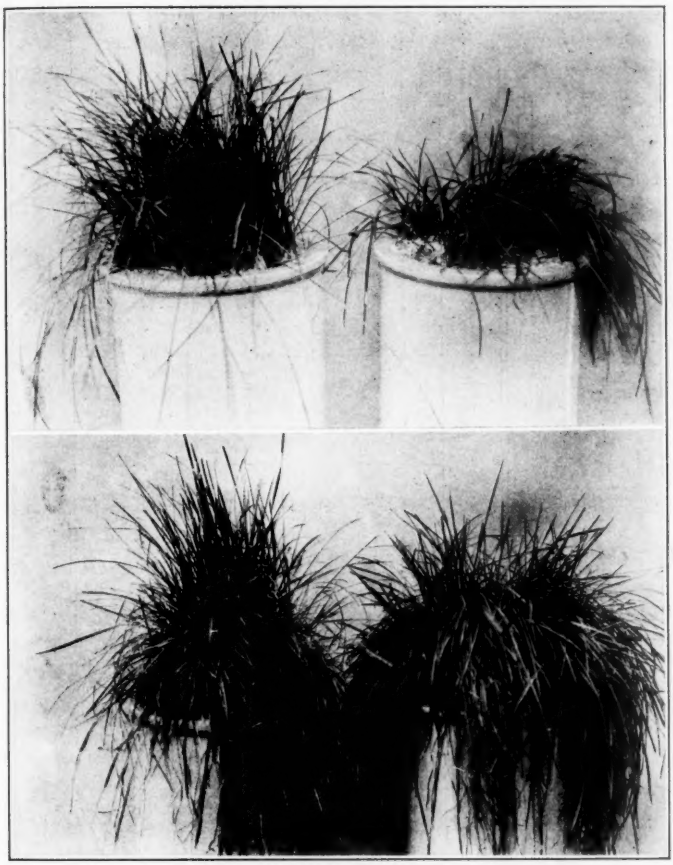


FIG. 3. Below: on the left a culture which had no nitrogen added in the nutrient solution but which had additional light after November 10 as compared with the one on the right which had a continual supply of nitrogen but no additional light. Photographed December 15; previous treatment identical.

Above: the same cultures 2 weeks after the last of three cuttings on December 15, 28, and January 6. Plants were cut back to approximately 1 inch.

days with cool temperatures the plants which had been cut and those left uncut appeared almost identical.

In order to note the amount of growth made by grass at different times of the year, cutting was continued on several of the cultures that were cut for the first time on September 22. Some were supplied with nitrogen continuously and some were given a solution containing no nitrogen. Table

TABLE III

DRY WEIGHT OF CLIPPINGS REMOVED FROM CULTURES WITH AND WITHOUT A CONTINUOUS SUPPLY OF NITROGEN AT VARYING INTERVALS OVER A PERIOD OF NINE MONTHS

CULTURE	CUT BACK TO	INITIAL CUT	SUCCESSIVE CLIPPING DATES						
		9/22/31	9/30/31	11/12/31	11/23/31	12/4/31	1/11/32	3/18/32	7/6/32
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
$+NO_3$	0.5 inch	29.0	1.32	5.0	0.4	0.4	0.1	3.1	42.6
	1 "	25.0	1.05	5.7	0.9	1.0	0.4	6.9	42.5
	2 "	23.0	1.05	6.7	0.8	0.9	0.2	8.1	44.7
$-NO_3$	0.5 "	29.0	1.20	3.55	0.33	0.15	0.02	Dead	.....
	1 "	40.0	1.95	4.50	0.35	0.25	0.15	1.0	4.1
	2 "	34.0	1.20	6.70	0.70	0.70	0.20	0.9	3.6
$+NH_4$	0.5 "	25.0	0.50	0.10	0.02	0.03	0.02	1.1	6.5
	1 "	25.0	0.70	0.90	0.15	0.23	0.08	2.7	9.7
	2 "	29.0	1.05	1.95	0.20	0.20	0.05	2.7	6.3
$-NH_4$	0.5 "	36.0	0.60	0.50	0.07	0.15	0.05	0.6	1.6
	1 "	37.0	0.40	0.60	0.10	0.20	0.10	0.7	1.2
	2 "	27.0	0.70	1.70	0.20	0.30	0.15	1.2	1.8

III shows the weights of the clippings removed from these cultures at different times during a period of approximately nine months. The data indicate that the addition of nitrogen when the days were short and cloudy had little effect on the amount of new top growth produced.

Experiment III

Effect of cutting plants with and without an added nitrogen supply during the summer months.—The effects of cutting two cultures with different treatments, one grown with a continuous supply of nitrogen and the other supplied a nutrient solution without nitrogen, during the winter months characterized by short, cool, cloudy days, have been detailed. Tests were then made on plants during summer conditions of long sunny days with high temperatures. The plants were started on November 11, 1931, from 6-gm. segments of the same original plant used in all the previous experiments. They were supplied with a complete nutrient solution containing nitrogen in the nitrate form only. On April 21, the sand in eight cultures was washed free of nitrates and then supplied with a minus-nitrogen solution by substituting calcium chloride for calcium nitrate. The regular complete solution containing nitrate was continued on eight other cultures.

It took approximately six weeks for the accumulated nitrates in the leaves of the plants, now receiving no nitrogen, to disappear. On June 20, two months after the minus-nitrogen treatment was started, three cultures which had been receiving nitrogen and three which had received no nitrogen were dug up, the sand washed from the roots, and the plants separated into roots, rhizomes, and tops. The dry weights of the plant parts are shown in table IV. The three plants were used to serve as checks one upon the other.

TABLE IV

Dry weight of roots, tops, and rhizomes from plants grown with and without nitrogen

Cultural conditions	No.	Roots	Tops	Rhizomes	Totals
		gm.	*gm.*	*gm.*	*gm.*
Plants grown without nitrogen for two months	1	16.3	41.7	20.6	78.6
	2	16.4	38.5	21.0	75.9
	3	20.1	46.5	27.6	94.2
Plants grown with nitrogen continuously	1	13.7	59.5	15.0	88.2
	2	14.0	64.7	13.2	91.9
	3	11.8	53.2	11.7	76.7

The average dry weight of an entire plant in the minus-nitrogen set was 82.9 gm., while that in the plus-nitrogen set was 85.6 gm. The dif-

ference in dry weight between the whole plants in the two treatments was very small, but the relative amounts of the different parts of the plants, roots, tops, and rhizomes, showed considerable variation. The usual preponderance of tops to roots in the cultures supplied continuously with nitrogen is shown.

On June 21, the remaining five of each set were cut back to 0.75 inch above the level of the sand in the pot. The cutting was continued once each week thereafter, the clippings collected, and the green and dry weights recorded. Table V shows the weights of clippings removed.

TABLE V

GREEN AND DRY WEIGHTS OF CLIPPINGS REMOVED FROM CULTURES WITH AND WITHOUT A CONT

CONDITIONS	No.	JUNE 21		JUNE 30		JULY 7		JULY 14		JULY 21		JULY 28		AUGUST 4	
		G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Cultures receiving continuous nitrogen supply	1	240	62.8	12.4	1.6	7.8	1.2	2.5	0.6	1.1	0.3	0.5	0.1	5	0.1
	2	192	49.2	10.1	1.5	5.8	0.9	2.6	0.6	2.8	0.8	1.3	0.4	4	0.5
	3	245	65.5	10.0	1.5	6.6	1.0	3.0	0.8	2.8	0.8	1.0	0.3	4	0.3
	4	235	61.4	10.6	1.6	8.6	1.4	2.8	0.7	1.9	0.6	1.4	0.5	4	0.3
	5	242	63.5	7.5	0.9	5.3	0.9	2.7	0.7	1.2	0.4	0.7	0.2	7	0.1
Cultures grown without nitrogen since April 21	1	121	38.2	5.1	0.7	3.2	0.6	2.9	0.9	1.5	0.5	0.9	0.3	2	0.5
	2	165	48.1	6.1	0.9	3.7	0.7	2.5	0.8	2.2	0.7	1.4	0.4	1	0.5
	3	153	47.7	5.1	0.7	3.5	0.6	1.6	0.5	2.4	0.7	1.4	0.4	3	0.5
	4	151	42.9	4.6	0.6	3.8	0.7	1.4	0.4	1.9	0.6	1.1	0.3	1	0.4
	5	173	53.9	5.5	0.8	4.5	0.8	1.8	0.5	3.9	1.1	2.0	0.6	2	0.5

It will be noted from table V that the cultures which received a continuous nitrogen supply produced considerably more tops, removed in the initial cutting, than those supplied with a minus-nitrogen solution. Also the clippings removed from these cultures each week, for the first three weeks after the initial cut, were greater in weight than the clippings from those receiving no nitrogen. On the average, however, those removed at the fourth cutting were greater in weight from the cultures receiving no nitrogen than from those which had had a continuous supply. For the following nine weeks this same balance in favor of the cultures receiving no nitrogen held true. The cultures supplied with nitrogen exhibited a very weak growth which was pale green in color. Those which had been growing without nitrogen started very slowly the first week after the initial cutting. They grew about 1.5 inches while the cultures supplied continuously with nitrogen grew 4 inches. The cultures without nitrogen changed in color from a yellowish green at the start of the test to a dark green which

was maintained until the close of the experiment. A considerable number of new stems appeared above ground in these cultures, while in those supplied nitrogen no new stem tips were visible.

A study of the underground parts of the cultures on June 30 showed no difference between the two sets of cultures; but on July 7, approximately two weeks after the initial cutting, a large number of the rhizome tips in the cultures supplied continuously with nitrogen were dead. In the cultures supplied with the solution containing no nitrogen there were no dead rhizome tips observable, but the growing points of the rhizomes were turn-

TABLE V

ND WITH T A CONTINUOUS NITROGEN SUPPLY DURING THE SPRING AND SUMMER MONTHS

JULY 28		AUGUST 4		AUGUST 14		AUGUST 21		AUGUST 28		SEPTEMBER 4		SEPTEMBER 12		SEPTEMBER 20		SEPTEMBER 27	
G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.
gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
		5	0.1	0.3	0.1	0.2	0.5	0.2	0.05	Dead							
		4	0.5	2.1	0.6	1.6	0.4	1.7	0.4	0.8	0.2	1.1	0.2	1.1	0.3	0.8	0.2
		4	0.3	1.2	0.3	1.0	0.2	1.0	0.2	0.7	0.2	1.2	0.2	1.2	0.3	1.2	0.3
		4	0.3	1.1	0.3	0.8	0.15	0.7	0.15	0.2	0.05	0.7	0.15	0.2	0.05	0.2	0.05
		7	0.1	0.3	0.1	0.3	0.05	0.2	0.05	0.1	Dead						
		2	0.5	2.1	0.7	1.7	0.5	1.7	0.4	1.1	0.4	2.4	0.5	2.2	0.6	1.4	0.5
		1	0.5	2.7	0.8	1.9	0.6	1.8	0.4	1.2	0.4	2.2	0.5	2.2	0.6	1.2	0.4
		3	0.5	2.7	0.8	1.9	0.6	1.6	0.4	1.1	0.4	2.2	0.5	2.1	0.6	1.5	0.5
		1	0.4	2.2	0.6	1.6	0.4	1.4	0.35	0.9	0.2	1.3	0.3	1.2	0.3	0.8	0.2
		2	0.5	2.6	0.7	1.8	0.5	1.6	0.4	1.2	0.3	2.5	0.5	2.5	0.7	1.5	0.5

ing upward, and a few were producing, in place of scale leaves, yellowish vegetative leaves which were emerging above the soil line. At no time did any new rhizomes or stem tips appear above ground in the cultures supplied continuously with nitrogen. On July 20, after four weekly cuttings (that is, one month after the initial cuttings), all of the rhizome tips and a considerable number of the whole rhizomes in the cultures supplied with nitrogen were dead. No dead rhizomes were found in the cultures that had had no nitrogen since April 21. A large number of the rhizomes in these cultures had emerged above the soil line and were actively producing vegetative leaves. Some of them branched somewhat underground, but on August 15 practically all that were below ground at the start of the test had emerged above the soil line. No dead stems were noticeable in these cultures. At the same date, in the cultures which were supplied with nitrogen continuously, practically all of the rhizomes had died before emergence; likewise the roots attached to them and about 60 per cent. of the above-ground stems present at the beginning of the test had died.

The yield of top growth was also influenced by temperature. During the week from July 21 to 28, characterized by bright days and high temperatures, the yield decreased considerably in both sets of cultures when compared with the previous week or the following one. The day temperatures during most of the week reached a maximum of 120° F. and the night temperatures rarely went as low as 80° F. The following week (July 28 to August 4) the day temperatures reached a maximum of 90° F. and the night temperatures frequently went as low as 65° F. The week from August 27 to September 4 was also a very hot week and the yields were approximately half of what they were for the following week, which was cool.

On September 27 the experiment was discontinued. Two of the five cultures which had had a continuous supply of nitrogen were completely dead and a third was practically so. Of the two remaining, approximately 50 per cent. of the original top growth was dead. The individual plants still remaining alive were very shallow-rooted. All the rhizomes in all five cultures, together with the roots attached to them, were dead. On the other hand, the cultures which had been grown since April 21 without nitrogen had many small roots still present and alive. These cultures were beginning to show a nitrogen deficit, as evidenced by yellowing of the leaves. A considerable number of rhizome tips were still present below the soil line and no dead tips were discernible.

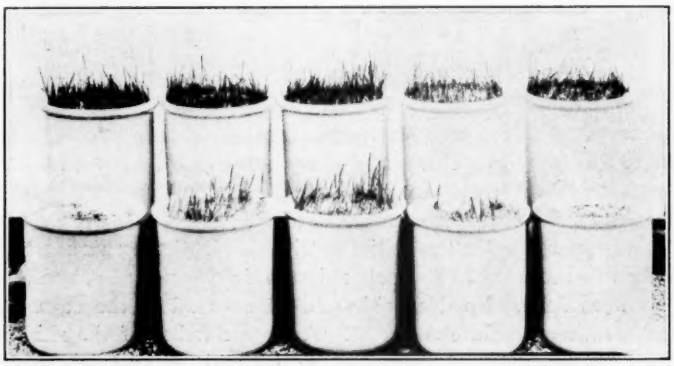


FIG. 4. Top row shows the five cultures which had no external nitrogen supply after April 21; bottom row shows the five cultures which had a continuous nitrogen supply. It will be noted that the two end cultures on the bottom row are completely dead.

EXPERIMENT IV

EFFECT OF CUTTING AND FERTILIZING ON GRASS GROWTH AT DIFFERENT TEMPERATURES.—It has long been recognized that Kentucky bluegrass

makes a better growth in cool weather than in hot. What effect would cutting at different temperatures have on the recovery growth of the grass? Will it grow faster at high temperatures than at low? How will rhizomes react to the different temperatures?

In order to answer these questions, some cultures which had been started on January 7 from 6-gm. segments were placed, on July 15, in constant temperature glass cases. All these cultures had received a complete nutrient solution, containing nitrogen in the nitrate form only, until May 30, after which they were supplied with a minus-nitrogen solution. The constant temperature cases were set at 60°, 80°, and 100° F. respectively. The temperatures in all of the cases varied slightly but never more than 3°. The humidity of the air was adjusted so that the saturation deficit would be approximately the same at all three temperatures. Eight cultures were placed in each case: four were supplied with a complete nutrient solution containing nitrogen in the nitrate form only, and four were supplied with a minus-nitrogen solution. The plants were left at these temperatures without cutting for five days. All of the cultures at 100° F. were beginning to turn more yellowish at the end of this period, whereas those at 80° and 60° which had been supplied with nitrogen were turning a dark green. No color change could be noted in the cultures receiving a minus-nitrogen treatment at these temperatures.

On July 20 the grass was cut back to approximately 1 inch and the weights of tops removed recorded. On July 24, nine days after the beginning of the test, it was observed that the rhizomes were dying rapidly in the cultures at 100° F. They appeared to be dying faster in the cultures supplied with nitrogen than in those not receiving nitrogen. No rhizomes had died in any of the other four sets of cultures. The cultures subjected to 100° F. grew very little either with or without nitrogen. The grass was again clipped back to the 1-inch height, ten days after the initial cutting

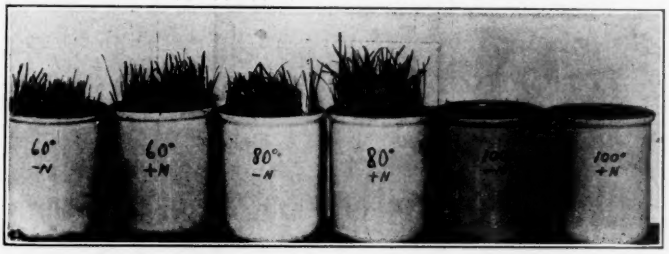


FIG. 5. Photograph taken July 27, showing the first week's recovery growth after the initial cutting on July 20 of cultures grown at three different temperatures, with and without nitrogen.

TABLE VI

AVERAGE GREEN AND DRY WEIGHT OF FOUR CULTURES AT 100°, 80°, AND 60° F. WITH AND WITHOUT NITROGEN

DATE	100° F. – N		100° F. + N		80° F. – N		80° F. + N		60° F. – N		60° F. + N	
	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.	G. WT.	D. WT.
(Initial cutting)	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
July 20	106.6	37.9	98.5	35.9	129.0	44.8	106.4	37.5	128.2	44.2	114.1	39.7
July 30	1.05	0.29	0.9	0.25	6.5	1.5	12.8	2.5	5.4	1.1	10.6	1.8
August 9	No growth				4.0	0.9	6.4	1.2	2.4	0.47	10.4	1.8
August 20	No growth				3.0	0.8	3.7	0.8	1.6	0.4	9.8	1.8
August 31	No growth				2.8	0.6	3.2	0.5	1.6	0.3	10.5	1.9
September 12	No growth				3.5*	0.8	3.7	0.7	1.9	0.4	10.9	1.7

* Average of three cultures. Some solution containing nitrogen was accidentally spilled into one of the four cultures so it was discarded.

and every ten days thereafter until the close of the experiment. The weights of the tops removed from each culture are recorded in table VI.

The cultures at 100° F. were discontinued August 31 as none of them were growing and all of the original tops appeared dead with the exception of two or three leaves in the minus-nitrogen set. The pots were left in the greenhouse and watered daily. The temperature reached a minimum of around 70° F. during the night, which was considerably cooler than the temperature to which they had been previously exposed. On September 12 it was noted that a few spindling light green leaves were appearing around the edges of all the cultures except one of the four which had been supplied with nitrogen. Upon closer examination it was observed that this growth was coming from some of the dormant lateral buds on a very few of the rhizomes, in no instance from the terminal bud.

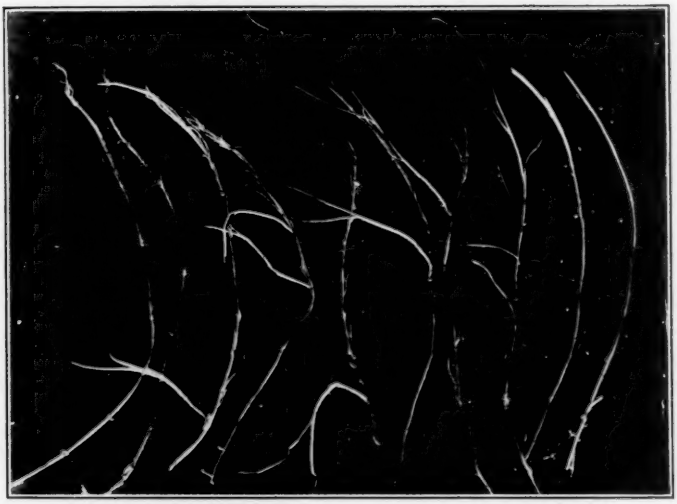
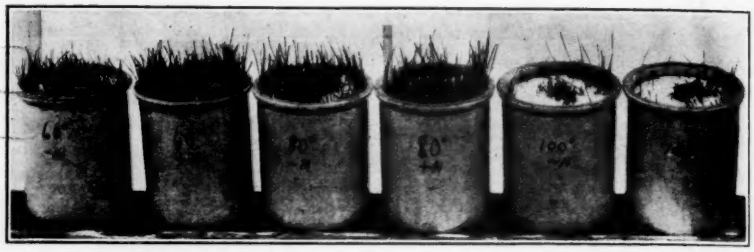


FIG. 6. On the left two rhizomes which were completely dead. The next five show the nature of the recovery growth from the rhizomes in the 100° F. cultures. Note the new growth back from the tip of the rhizome and the dead portion above it. Next to these five is shown a rhizome which had just emerged above the soil line and was producing vegetative leaves. The last two on the right show the appearance of the rhizomes underground and producing only scale leaves.

At the end of the test the cultures were separated into living tops, dead tops, roots, and rhizomes. No attempt was made to separate the dead underground tissue from the living portions. Table VII shows the weights of the different portions at the close of the test.



To replace Fig. 7, page 100 in the January number of PLANT PHYSIOLOGY.

FIG. 7. Photograph taken September 20 showing nature of the top growth produced during the last week of the test. Note the thickening up of the grass in the cultures at 60° + N and the spindling growth in the cultures exposed to 100° until August 31.

TABLE VII

DRY WEIGHT OF PLANT PARTS AT END OF TEST (AVERAGE OF FOUR CULTURES)

PARTS	100° − N	100° + N	80° − N	80° + N	60° − N	60° + N
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Roots	10.5	9.9	12.2	9.3	16.4	11.2
Rhizomes	6.1	7.0	7.4	7.7	12.9	9.2
Living tops	0.25	0.16	7.7	2.8	8.4	12.3
Dead tops	4.5	4.5	1.6	4.9	None	None

Table VII shows that the roots and rhizomes in the cultures at 60° without nitrogen weighed far more at the conclusion of the test than those of any of the other cultures; also, that there were no dead tops whereas there were many at the other two temperatures. Obviously either high temperatures or the addition of nitrogen may bring about a decrease in the weight of the underground storage portions of the plant.

It will be observed from figure 5 and table VI that the grass made very little recovery growth either with or without nitrogen at 100° F. After the second cutting these cultures did not produce any new top growth until after the exposure to 100° F. was discontinued. The tips of the rhizomes were practically all dead in both sets and very little green top growth was noticeable. At 80°, the production of new leaves after cutting was very rapid when nitrogen was added and much slower in the minus-nitrogen treatment. The leaf blades in the cultures without nitrogen were short and broad, while those receiving nitrogen were long and narrow. None of the rhizomes present at the beginning of the test had begun to appear

in either set. On closer examination, it was observed that the tips of a number of rhizomes were dying underground in the cultures receiving nitrogen, whereas those supplied with a minus-nitrogen treatment were turning upward and growing toward the surface of the sand. The weight of the clippings removed after the first ten days from the cultures receiving a minus-nitrogen treatment was approximately one-half of that removed from the cultures receiving nitrogen. One month later the clippings removed were approximately the same in both sets, with or without nitrogen. At 60°, in the cultures supplied with nitrogen, the first observable change was the emergence of a large number of rhizome tips, which were underground at the beginning of the test. These had grown rapidly upward through the sand and emerged into the light. Tiller-like buds at the base of the leaves of the older above-ground stems were also beginning to grow and produce vegetative leaves. The top growth of these cultures, which consisted primarily of upright leaf blades, became considerably denser, and although the grass did not grow so tall as it did at 80° with nitrogen, the weight of the recovery growth was almost equal. One month after the initial cutting, the figures show that the cultures supplied nitrogen at 60° were producing approximately three times as much weight of tops as the cultures receiving nitrogen at 80°. This difference was largely due to the increased number of new, above-ground stems in the cultures at 60° which were producing leaves. At 60°, when the cultures were supplied with nitrogen the rhizomes grew upward into the light, while at 80° they died before emerging and no new tiller-like buds were produced by the older above-ground stems. Much more green leaf tissue was left after cutting in the case of the grass in the cultures at 60° supplied with nitrogen than at 80°, because these new stems were green on emergence and had considerable leaf area left below the cutting point; whereas most of the green tissue was removed from the cultures at 80°. The cultures at 60° without nitrogen very slowly produced new top growth following cutting, and upon closer examination it was noted that these plants were actively producing new roots, a characteristic not observable in any of the other sets of cultures. Even after the fourth successive cutting these cultures were still producing new roots.

Summing up the general observations on the effects of the variations in temperature, the following points can be noted: (1) at 60°, minus nitrogen, the top growth recovery following cutting was slow, none of the rhizomes pushed out above the soil, and the cultures produced many new roots; (2) at 60°, plus nitrogen, no new roots were produced but many of the rhizomes below ground at the beginning of the test turned upward and pushed above the soil line; (3) at 80°, minus nitrogen, no new roots were produced and some of the rhizomes gradually appeared above ground; (4) at 80°, plus

nitrogen, no new roots were produced, no rhizomes appeared above ground, and a large number of rhizomes and roots died; (5) as the number of cuttings increased, the yield of tops between cutting periods in the cultures receiving the treatment of minus-nitrogen at 80° was beginning to approximate the yield of those receiving nitrogen; (6) at 100°, in either set there was very little new top growth and the rhizome tips and the roots practically all died.

A final consideration of the results of the various experiments, as set forth in the foregoing tables and illustrations, brings out several characteristic responses of Kentucky bluegrass to changes in temperature, amount and intensity of light, variations in nitrogen supply, and cutting practices.

Tables II and III and figure 3 show that bluegrass did not grow the same in winter when the days were short and cloudy as it did in summer when the days were long and sunny. The plants produced the largest amount of new tops, roots, and rhizomes during the spring and fall when the temperatures were not excessively hot or cold, and the days were bright and long. The plants, during the long, sunny days of spring, summer, and fall, produced a large number of rhizomes if continuously fertilized with nitrogen and left uncut; but as the short cloudy days of winter arrived, these rhizomes turned upward and emerged into the light above the soil line. If the nitrogen supply was discontinued during this period of short, cloudy days, the rhizomes not only remained below the level of the ground but became stockier and increased in number. Severe cutting of the tops of a culture continuously supplied with nitrogen was much more disastrous during this short cloudy period than during the period of long, bright days of spring, early summer, and early fall. If the days were long and sunny but characterized by excessively high temperatures, the cultures produced less new growth between cuttings than they did when the temperatures were lower.

On the other hand, close and frequent cutting of a plant that had been growing for some time without an external supply of nitrogen and the leaves of which had turned from green to yellow did not bring about the death of any of the above-ground stems, roots, or rhizomes. This plant had a large number of thick, tough rhizomes; the plants continuously supplied with nitrogen and grown during the period of long sunny days had fewer and more succulent rhizomes; whereas the cultures grown with a continuous supply of nitrogen during the winter had none. The rhizomes of the latter had either come up to the surface or died before emergence. It seems evident that the rhizomes may store a supply of carbohydrates which may be used by the plant during periods of short and frequent clipping or during periods of dark, cloudy weather. When the plants were kept extremely vegetative very few rhizomes were formed, and when in this

condition, close and frequent defoliation resulted not only in lessened production of new leaves but actually caused death of the stems above ground, and of roots and rhizomes.

The plants which were grown in the winter period of dull, cloudy days, and which received a continuous supply of nitrogen, had practically no rhizomes and therefore had very little underground storage supply of carbohydrates. If the tops of these cultures were kept cut off continuously the plants died very soon, while others without nitrogen and which had a large number of thick, sturdy rhizomes continued to produce new top growth; and none of the roots, rhizomes, or above-ground stems died during the course of the experiment. As the cutting continued, the rhizomes of the plant without nitrogen gradually turned upward, and emerged above the soil line and produced mainly vegetative leaves. The amount of defoliation that a grass plant will survive is largely dependent upon the rate at which the carbohydrates, stored in the underground portions of the plant, are used up. When the plant is completely defoliated, the drain upon the reserve carbohydrates is much more severe than it is when considerable leaf area remains after cutting. When temperatures are between 70° and 90° F., and there are sufficient moisture and nutrients, the new leaves produced grow quickly and rapidly. The constant removal of this new growth soon brings about the death of these plants through carbohydrate starvation, whereas with constant cutting a plant with high carbohydrate storage and without an external supply of nitrogen grows slowly during the periods between cuttings, thus utilizing smaller amounts of the stored carbohydrates and continuing growth over a longer period of time. In the case of the vegetative plant, a large amount of top growth is removed at each cutting; but such cutting can continue only over a short period of time, after which the development of new leaves becomes slower and slower until the plant in many cases actually dies. In contrast to the vegetative plant, a small amount of top growth is removed from the non-vegetative plant at each cutting, but the production of new leaves continues over a much longer period of time.

These results as a whole indicate that fertilization of grass with nitrogenous fertilizers during periods of short and frequent cutting at high temperatures does not result in an increase of growth but may even result in a decrease. Several factors, such as short and frequent clipping, shade, short cloudy days, nitrogen fertilizers, heavy watering, and high temperatures tend toward the using up of such supply of stored carbohydrates as may be available, and if carried to such an extreme that these carbohydrates are completely utilized and are not made available through the activity of additional leaf area, ultimate death of the plant may be brought about. Short and frequent cutting and fertilizing with nitrogen may help consid-

erably in the production of a denser turf provided the plants have a carbohydrate reserve, or a ready means of its manufacture, and the weather remains cool and bright.

Summary

1. The leaf blades of bluegrass cultures supplied with calcium nitrate were shorter and more upright, the rhizomes were more numerous, stockier, and more branched than was the case when the plants were supplied with ammonium sulphate.

2. The rhizomes and a large proportion of the roots of vegetative cultures were killed by short and frequent defoliations.

3. The plants exhibited a different type of growth at different seasons of the year, when light conditions, nitrogen supply, and cutting practices were varied. Rhizomes produced in cultures left uncut during the bright cool days of spring, early summer, and early fall and continuously supplied with nitrogen, gradually emerged into the light as the short cloudy days of late fall and winter arrived. By limiting the nitrogen or increasing the light, these rhizomes not only remained below the soil level but became stockier and new ones were also produced.

4. It was possible to influence the amounts and relative proportions of various plant parts by limiting the nitrogen supply. As the nitrogen supply was decreased, the relative amount of top growth diminished as the roots and rhizomes increased.

5. Cutting back the leaves of non-vegetative plants supplied with a minus-nitrogen nutrient solution, and which had a large quantity of storage rhizomes, was less harmful during the hot summer months than was short cutting of vegetative plants which received a continuous supply of nitrogen and which had a much smaller quantity of storage rhizomes. It was possible to kill plants that were continuously fertilized with nitrogen by frequent defoliations.

6. Plants grew very little at 100° F., after defoliating, with or without nitrogen; and a large part of the established cultures died during six weeks of such exposure. A few dormant buds on the rhizomes recovered after the exposure was discontinued and the temperature lowered. In no case was such recovery from a terminal bud on the rhizome but rather from a bud several nodes back from the tip.

7. When cultures exposed to 80° F. and supplied with nitrogen were periodically defoliated, new leaves were produced which grew rapidly. This rapid growth exhausted the reserve supply of carbohydrates during the first few periods; and as the defoliations were sufficiently frequent to prevent a replenishing of the supply, the growth made between cuttings became less and less. Finally these cultures were producing no more new

growth between the periods of defoliation than were cultures exposed to the same temperature but supplied with a minus-nitrogen solution. The tips of the rhizomes in the cultures supplied with nitrogen died, while the rhizomes in those receiving no nitrogen turned slowly upward and emerged above the soil line.

8. At 60° F., when the cultures were supplied with nitrogen the new leaves produced after a defoliation grew much more slowly than was the case at 80° F., but soon practically all of the rhizomes present at the beginning of the test had emerged above the soil line, and tillered profusely on emergence; and these cultures, because of this different habit of growth, were soon producing a much higher yield of new growth between defoliations than were the cultures at 80° F.

9. At 60° F., without nitrogen, the response to close cutting was the production of many new roots. During the course of the experiment the rhizomes remained below ground and no dead tips were discernible. The cultures yielded very little new top growth during the periods between defoliations.

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HYDRATION OF SOLUTE IONS IN RELATION TO ACIDITY, ALKALINITY, AND pH

LEWIS H. FLINT

Introduction

During the last few years an increasing amount of research has been directed to the solution characteristic called "acid" or "alkali," and more recently interpreted as a concentration of H^+ ions and expressed as pH. On the one hand this attention has been accompanied and fostered by an increasing recognition of the importance of the characteristic in the precise control of varied solution reactions in industrial processes, and in its practical application to such processes as measured by colorimetric or electrometric methods, it has been widely accepted as a means of standardization and control. On the other hand the attention has given rise to divergent interpretations of the factors responsible for the characteristic. The view embodied in such treatises as those of CLARK (**9**), MICHAELIS (**18**), and BRITTON (**7**) is that the concentration of H^+ ions is responsible for the attribute. Yet GORTNER (**14**) and others have found no satisfactory assurance in this interpretation, and have substituted "ionic activity" for "hydrogen ion concentration" in explanation. The possible effectiveness of other than H^+ ions has thus been recognized.

During the last few years also an increasing divergence of opinion has developed in regard to the state of dissolved substances. Following the wide acceptance of the ionization theory of ARRHENIUS, there came a wide acceptance of the theory of incomplete dissociation, a theory developed largely in explanation of the more or less parallel decreases in specific molecular electrical conductivities and freezing-point depressions with increasing concentrations. During the régime of the wide acceptance of this theory most of our present texts were written, so that we may look upon it as the orthodox system. Yet with an increasing amount of evidence pointing to complete ionization at all concentrations, a situation has arisen in which, on the one hand, many investigators take complete ionization for granted, while on the other hand many others assume incomplete ionization. Still more recently others have sought to build on the DEBYE-HÜCKEL theory as a sort of mathematical compromise involving electrical forces.

Whatever view-point one has acquired with respect to the validity of the pH interpretation of acidity or with respect to ionization, however, it is inevitable that the increasing differences of opinion in regard to these matters further complicate the direction of research in solutions. From the study of pH measurements involving controllable amounts of known materials, we may obtain ample assurance that these measurements afford a

means of marking a definite and reproducible stage in various processes. The measurements are thus of great practical value, independent of the validity of any interpretation. From the study of pH measurements involving unknown amounts of unknown materials, however, we obtain little assurance. It appears that the same pH may characterize both a favorable and an unfavorable medium; that the pH of an organism may be modified by the medium or the pH of the medium may be modified by the organism; and that the amount of buffering material in either the organism or the medium may condition the measurement. Under these circumstances it appears that we should not unhesitatingly accept the postulate that solute ions other than H^+ ions (or H^+ and OH^- ions) have little or no influence on the pH measurement. Yet if other ions do influence the measurement, what is the manner of their action and how may we evaluate it? The subject is a precarious one, for the widely recognized usefulness of the pH measurement in marking the progress of industrial processes has fostered the acceptance of the H^+ ion interpretation; and any departure from this interpretation must meet a great deal of criticism. On the other hand, since the acceptance of the H^+ ion interpretation appears inconsistent with many observational data of physiological and physico-chemical research, we are not honest with ourselves if we do not venture a reconsideration of the significance of the pH measurement.

The conventional interpretation of the pH measurement was developed during the wide acceptance of the theory of incomplete ionization, and the resourcefulness of its exponents in the field of explanation would have been wholly commendable if the results obtained had led to a position from which other solution characteristics could have been predicted. The interpretation was based, obviously enough, upon the theory of incomplete ionization, and is so involved with that theory as to be untenable in its present form if associated with the theory of complete ionization.

During more recent years, however, as has been pointed out, an increasing body of evidence in support of complete ionization has been forthcoming. This evidence cannot be ignored, nor can we remain oblivious to the fact that this evidence is contrary to the conventional H^+ ion interpretation of pH measurements. Suppose, moreover, that important additional evidence in support of the theory of complete ionization was forthcoming, how would we then interpret the pH measurement?

Important additional evidence in support of the theory of complete ionization at all concentrations has been developed recently by the writer (**11, 12, 13**) from an extension of GRAHAM'S law to solute ions through the assumption of a periodic inverse integral hydration. There may be many not yet ready to acknowledge the admissability of this evidence because it involves the seemingly radical assumption of weight change with ioniza-

tion. It nevertheless becomes apparent that the specific hydration of solute ions not only permits the demonstration of complete ionization with the very data of electrical conductivity and freezing-point depression which were so largely responsible for the development of the theory of incomplete ionization, but, what is more important, permits the prediction of many solution characteristics at all concentrations. Heretofore we have never had a solution theory which proved at all satisfactory in concentrated solutions. Naturally there can be no question but that the new view-point will have its own limitations in its application to solution phenomena, but the extent to which it has already lent itself to prediction appears to warrant the study of its possible significance in relation to various phases of solution phenomena. At this time one of the most studied of these phases is acid-alkali reactivity. If the conventional interpretation of pH is the correct one, surely the relationship between solute and solvent will emphasize that correctness. If the conventional interpretation of pH now seems erroneous, however, let us cheerfully acknowledge our past mistakes and set about making our readjustments to a modified view-point with that zest which has always made research high adventure. Moreover, if our studies revive the ghost of PROUT and hold out a wholesome simplicity to chemical science, let us not flinch before the academic prestige of the printed page and inherited interpretations. The chemist with the modern concept that ideas must be complex in order to be valid will not be interested in this paper. Yet as physiologists may we not be permitted to acknowledge our disappointment in the conventional interpretation of the pH measurement and to question its correctness? When we lose the power and courage to face the facts and think independently we will have become useless for research, and may as well turn our work over to regimented robots.

The new description of hydration

There may be those who have some misgivings in regard to the existence of an attractive force between solute and solvent which is termed hydration, but in general such a force has long been widely recognized. JONES and his associates (**15**) took an important part in bringing about this recognition through the establishment of several independent lines of evidence all pointing to the existence of hydrates in solution. Whether or not one accepts their further description of the relationship (and it now appears demonstrable as invalid), one may scarcely venture to deny the existence of the attractive force, particularly since various other researches during the last half-century have similarly indicated its existence.[1] Without desiring to seem arbitrary in this matter, it seems best not to complicate the

[1] A valuable series of papers on hydrates in solution will be found in Trans. Faraday Soc. 3, part 2. Oct. 1907.

discussion at this point with a comprehensive examination of the evidence for hydration, but rather to accept the findings of these various workers to the extent of subscribing to the existence of an attractive force between solute and solvent, restricted, of course, to those solutes and solvents in which the force has been so thoroughly demonstrated. A detailed study of the researches on hydration leads almost inevitably to the conclusion that such a view-point is justified.

With particular reference to acidity and alkalinity, however, it is to be noted that the researches on hydration demonstrate the existence of little or no attractive force between an H^+ or an OH^- ion and an aqueous solvent (**15, 22**). These ions are not the only exceptions, for the somewhat analogous SH^- and S^{--} ions appear similarly characterized; but it is of major concern to us that the H^+ and OH^- ions seem to be outstanding representatives of a small group in which practically no attraction of the sort designated as hydration can be demonstrated between the ions and their aqueous solvent.

Against the background of solute ions for the most part characterized by an attraction for their aqueous solvent, yet with a few characterized by the absence of such an attraction, we may now take up the more detailed description of the attraction called hydration as applied to dissolved substances. There have been a number of attempts to extend the description of hydration beyond the abstract status of an unevaluated force, and it may be well worth while to review these studies at this point. Yet to bring something of conciseness to such a review it seems best to select an electrolyte as representative and standard. The electrolyte KCl in aqueous solution is the conventional standard for studies of the electrical conductivity of solutions, and appears suitable to serve likewise as a standard for studies of hydration. We may now turn to the experimental data, therefore, and examine the attraction there indicated as existing between the solute KCl and its aqueous solvent.

Using the depression of the solubility of a gas accompanying the addition of an electrolyte as an index of the amount of bound water, or water associated with the electrolyte through hydration, the results summarized in table I were obtained by KNOPP (**16**), STEINER (**23**), PHILIP (**20**), and GORDON (cited by ARMSTRONG and CALDWELL **1**).

The results given in table I indicate that, on the basis of the method there represented, about ten molecules of water are associated with each molecule of KCl in aqueous solution. The results obtained by other methods are in less satisfactory agreement among themselves, but nevertheless of interest. BOUSFIELD (**4, 5**), working with observed relative migration velocities of ions as indices of hydration, obtained 12 as the specific molecular hydration of KCl in his earlier work, and 9 in his later work,—values of the order in-

TABLE I

SPECIFIC MOLECULAR HYDRATION OF KCl AS INDICATED BY THE EFFECT OF THIS SOLUTE UPON REDUCTION OF SOLUBILITY OF GASES IN SOLVENT

PERCENTAGE KCl BY WEIGHT	INVESTIGATOR	° C.	AVERAGE SPECIFIC MOLECULAR HYDRATION (NUMBER OF WATER MOLECULES ASSOCIATED WITH EACH MOLECULE OF SOLUTE)
1.09	KNOPP	20	9.8
2.12	KNOPP	20	11.1
3.83	STEINER	15	10.5
4.07	KNOPP	20	10.0
6.37	KNOPP	20	10.0
7.38	KNOPP	20	10.0
7.45	PHILIP	15	9.4
7.45	GORDON	15	9.9
7.48	STEINER	15	9.4
		Average	10.011

dicated in table I. CALDWELL (**8**), working with the accelerating influence of salts on actions in which acids were concerned, obtained 10 as the specific molecular hydration of KCl, a value in excellent agreement with the values in table I. In the work of six investigators using three methods there is thus to be observed a substantial concurrence of evidence that in an aqueous solution of KCl ten molecules of water are associated with each molecule of solute. Other results appear widely scattering. JONES and his associates (**15**), from a study of so-called "abnormal" freezing-point depressions, concluded that there was no hydration in solutions of KCl; while REMY (**21**), from considerations of supposed ionic radii in conjunction with observed ion mobilities, obtained 32 as the specific molecular hydration of KCl in aqueous solution. Obviously the methods used by these investigators must be held open to question, and until contrary additional evidence is brought forward we may scarcely do better than to conclude that 10 molecules of water are associated with each molecule of KCl in aqueous solution. It is important to note that this value is a whole number, and it will be recalled that the number of molecules of water observed as characterizing hydrated salts crystallizing out of aqueous solutions is usually likewise subject to expression in whole numbers per salt molecule.

The observational data relating to the specific molecular hydration of KCl will scarcely permit further conclusions at this point, but some additional findings are suggestive and of interest. ABEGG and BODLÄNDER (**2**) pointed out that, restricted to salts giving rise to a common negative ion,

hydration is inversely related to the atomic weight of the positive ion. This relationship was further corroborated and emphasized in the work of SENTER (**22**), BOUSFIELD (**5**), and JONES (**15**). BOUSFIELD further pointed out that the specific molecular hydration of the Cl^- ion is greater than that of the K^+ ion, and that the specific molecular hydration of the Na^+ ion is about four times that of the K^+ ion. There can be no doubt but that these investigators recognized the importance of the foregoing relationships, in particular the inverse relation between hydration and atomic weight, italicized in the work of JONES. The relationships, nevertheless, remained sterile until they became incorporated in the extension of GRAHAM'S law to solute ions and contributed to what may be called the new description of hydration.

Under the gas diffusion law of GRAHAM the velocity of a molecule is described as inversely proportional to the square root of its density:

$$V_1 : V_2 :: \sqrt{D_2} : \sqrt{D_1}$$

Combining this with the AVOGADRO gas law:

$$MW_1 : MW_2 :: D_1 : D_2$$

we have

$$V_1 : V_2 :: \sqrt{MW_2} : \sqrt{MW_1}$$

or the velocity inversely proportional to the square root of the molecular weight, a familiar and widely recognized relationship. Extending this relationship to solute ions we have

$$V_1 : V_2 :: \sqrt{IW_2} : \sqrt{IW_1}$$

or the velocity inversely proportional to the square root of the ionic weight. JONES (**15**) stated it as an established fact that the velocities of the ions were an inverse function of their mass, while BREDIG (**6**) found that the conductance of an element ion was a periodic function of its atomic weight. Applying the inverse relationship to the observed ionic mobilities of the K^+ and Na^+ ions (for example, at 18° C. $K^+ = 64.5$, $Na^+ = 43.3$ (10)) through the tentative addition of whole numbers of water molecules to these ions, and incorporating the observed specific molecular hydration observed for KCl, and also the suggestive additional findings above noted, we eventually derive an inverse *integral* relationship between ionic weight and hydration. Since the integral feature becomes incorporated, it follows inevitably that the ionic weights must have a corresponding regularity not afforded by the observed atomic weights, but rather such as that obtained by doubling the atomic numbers. These developments, though perhaps somewhat abstruse

as here abbreviated, and certainly unconventional however presented, really become illuminating when the observed ionic mobility of the H^+ ion, considered as unhydrated, is brought into the system; for it then becomes obvious that the basic weight assigned to the element through doubling its atomic number has been modified incident to, and in a direction depending upon, the ionization. The observed ionic mobility of the K^+ ion thus becomes evidence, under GRAHAM'S law and the foregoing observed relationships, that the K^+ ion of weight 2×19 (A. N.) $+2$ (positive charge), or 40, hydrates with 3 water molecules and moves as though of weight 94 ($40 + 3 \times 18 = 94$), and that the Cl^- ion of weight 2×17 (A. N.) -2 (negative charge), or 32, hydrates with 7 water molecules and moves as though of weight 158 ($32 + 7 \times 18 = 158$).

Unless one is in a position carefully to work out for himself the seemingly involved mathematical considerations introduced in the foregoing extension of GRAHAM'S law, it may be difficult to attain assurance of the integrity of the above interpretation, since there are a number of new ideas involved, some of which appear disturbing to the view-point to which we have long been accustomed. Moreover, in such a procedure many investigators would soon feel the need of a more detailed description than can possibly be included herewith in a paper on acidity and alkalinity. But after all the important thing about a description is not how one derived it, but what can it do; so we may proceed to outline some of the end results of the new description in so far as they immediately involve our base value ions, K^+ and Cl^-, and then venture prediction, the ultimate and only satisfactory test of any description being its serviceability in such a capacity. The new relationships embodying the weight range of the lighter elements are brought together in table II.

It may be observed that table II embodies most of what is known about hydration in relation to the involved elements. The associated water is expressed in whole numbers, analogous to the situation in hydrated salts crystallizing out of aqueous solutions. The associated water is also inversely related to atomic weight, noted and reported by several investigators as an outstandingly important relationship. The water associated with KCl totals 10 molecules, the value noted in the data of table I. The Cl^- ion with 7 water molecules is more hydrated than the K^+ ion with 3, as observed by BOUSFIELD, and the hydration of the Na^+ ion, 11 water molecules, is about four times that of the K^+ ion, 3 water molecules, as also observed by BOUSFIELD.

In proceeding to test the validity of the relationships represented in table II, it should be borne in mind that the values have been derived through studies of solute ions and thus pertain only to solute ions on the basis of observed measurements considered up to this point. It will readily

TABLE II

WEIGHT, HYDRATION, AND VELOCITY VALUES CALCULATED FOR THE LIGHTER ELEMENTS THROUGH AN EXTENSION OF GAS LAWS IN RELATION TO OBSERVED ELECTRICAL CONDUCTIVITIES

A.N.	E.	Assumed as weight 2 × A.N.	Velocity if not hydrated V_1	Postulated number of water molecules	Molecular weight of hydrated ion	Velocity if hydrated V_2	Ionic weights and velocities of the common hydrated ions			
							Ion	Weight		Velocity
								Anhydrous	Hydrated	Hydrated
0	.....	0	.....	23	414	491				
1	H	2	7082	22	398	501				
2	He	4	5000	21	382	512				
3	Li	6	4090	20	366	523				
4	Be	8	3546	19	350	535	Li^+	8	350	535
5	B	10	3162	18	334	547				
6	C	12	2887	17	318	561				
7	N	14	2672	16	302	575				
8	O	16	2500	15	286	591	F^-	16	286	591
9	F	18	2357	14	270	609				
10	Ne	20	2236	13	254	627				
11	Na	22	2132	12	238	648				
12	Mg	24	2041	11	222	671	Na^+	24	222	671
13	Al	26	1961	10	206	698				
14	Si	28	1890	9	190	726	Mg^{++}	28	190	726
15	P	30	1826	8	174	758				
16	S	32	1768	7	158	796	Cl^-, Al^{+++}	32	158	796
17	Cl	34	1715	6	142	839				
18	A	36	1667	5	126	891				
19	K	38	1622	4	110	953				
20	Ca	40	1581	3	94	1031	K^+	40	94	1031
21	Sc	42	1543	2	78	1133				
22	Ti	44	1508	1	62	1271	Ca^{++}	44	62	1271
23	V	46	1474	0	46	1474				

become apparent that, in the event these relationships are satisfactorily substantiated, the conventional observed weight data become subject to reexamination for an alternative interpretation; but this aspect is entitled to individual consideration and will be reserved for such treatment. For the present, therefore, we shall use the new weight values without apology, but leave the reader free to substitute the conventional observed weight values at his own risk.

We may establish our basic values for the electrolyte KCl in aqueous solution most readily from table II by selecting 1000 grams as the unit of solution and one gram molecule as the unit of solute. On this basis we derive

$K^{\circ} = 38$,	$K^{+} = 39$,	$K^{[+]} = 40$	hydrates with 3	$Cl^{[-]h} = 158$	$K^{[+]hv} = 1031$
$Cl^{\circ} = 34$,	$Cl^{-} = 33$,	$Cl^{[-]} = 32$	hydrates with 7	$K^{[+]h} = 94$	$Cl^{[-]hv} = 796$
KCl 72	72	72	10	252	1827

The exponential zero above signifies the neutral atom. In so far as we are aware such an atom does not exist in solution; probably it does not exist in the solid state, certainly not in the KCl crystal; and possibly it does not exist at all as a stable condition. Nevertheless the concept is inevitable under weight change with ionization. In the case of the Cl atom above, for example, the ionic form Cl^{+++++} preceding the formation of the radical ClO^{-}_{3} must be considered as having had a combining weight of 39; and at one point in the transition from the atomic state Cl^{+++++} to Cl^{-} there must have been neutrality. In a substantial measure the irregular observed weight values appear to have resulted from the failure to recognize weight change with ionization, and the consequent attempt to make one value represent the activity of various ions; but that is another story.

When we are forced to conclude that weight change accompanies ionization, therefore, we obviously mean that we must consider such a change simply as an adjunct of being dissolved, not, as might be thought, the change attendant upon the addition of a discrete particle. In the compound KCl, for example, the K^{+} and Cl^{-} ions are to be conceived as sharing a single electron (or a pair, if one prefers, depending upon the assigned weight values for the change) by virtue of which sharing the *combining* weight of the K^{+} ion, represented in the preceding discussion simply by an exponential plus sign, becomes subject to evaluation as 39, while the combining weight of the Cl^{-} ion becomes subject to evaluation as 33. Upon release through solution, however, we have an increase in the effective positive charge in the K^{+} and a corresponding increase in the effective negative charge of the Cl^{-}. Weight and positive charge thus become synonymous, a relationship which conventional chemistry denies to all elements except possibly the elements carbon, oxygen, and helium.

Weight now becomes resolvable into three categories: (1) neutral weight, not subject to measurement as yet; (2) combining weight, subject to measurement through combining ratios; and (3) ionic weight, subject to measurement through velocity under GRAHAM'S law. It is this ionic weight as indicated by velocity that has given us the relationships of table II, and, conversely, it is velocity predicted by expected ionic weight that is to become of interest in relation to acidity and alkalinity. The ionic state in this paper is designated by a bracketed exponent, followed in the case of hydration by a small *h* but otherwise signifying no hydration. The velocity is designated by an added small *v*.

From the preceding data relating to a specified solution of KCl we may venture to calculate a number of its expected characteristics. The weight of the hydrated solute being 252 grams with the total weight 1000 grams, it follows that the difference, 748 grams, is solvent. At this concentration, therefore, the weight of solvent is 74.8 per cent. of its weight at a zero concentration of solute. If this hitherto unevaluated change in concentration associated with hydration is correct, then the apparent decrease in specific molecular electrical conductivity and freezing-point depression, associated with an increase in concentration and interpreted as evidencing incomplete ionization, becomes attributable to such a change in concentration if the decrease is predictable on the new basis. If the observed values become constant following the correction for such a change, moreover, it follows that complete ionization must characterize the solution. In view of the increasing amount of evidence indicating such a complete ionization, we may venture to interpret the apparent rate of decrease in specific molecular electrical conductivity and freezing-point depression as an index of hydration. Conversely we may venture to predict, on such a basis, the apparent rate of decrease. Reference to observed data for aqueous solutions of KCl will reveal that the observed decreases in specific molecular electrical conductivity and freezing-point depression for solutions corresponding in concentration to 1.0 molecular, as previously described, are of the order of 74.8 per cent. (**3, 19, 11**). The conclusion is inevitable that hydration brings about an increase in concentration, and that the failure to recognize and correct for this increase in concentration contributed to the formulation of the theory of incomplete ionization.

From the foregoing data on KCl we may also venture to predict other solution characteristics, including the specific gravity, the capacity for gas absorption, and the transfer numbers of the ions. Yet in view of the results cited previous to the extension of GRAHAM'S law and the results just noted, there seems to be no real point in the further demonstration of the hydration characteristic of the solute ions K^+ and Cl^-. This is particularly true in that the velocity values for the hydrated K^+ and Cl^- ions, and the sum-

mation of these values, will be subsequently employed in considering some of the more dynamic aspects of solution phenomena.

Having already devoted considerable space to the manner in which the values of table II may be used in demonstrating and predicting static phases of solution phenomena, the reader must be left to satisfy himself with respect to the corresponding treatment of aqueous solutions of NaCl, LiCl, KF, NaF, $CaCl_2$, $MgCl_2$, and $AlCl_3$, all of which will be found substantiating the inverse integral hydration system (**3, 19, 11**). We may next turn our attention to the dynamic phases of solution phenomena, using an aqueous solution of KCl as a basis for the consideration of the velocity values derived from the extension of GRAHAM'S law in connection with the inverse integral hydration thus substantiated.

Velocity of solute ions in relation to acidity, alkalinity, and pH

In the foregoing discussion we have dealt with hydration in its effect upon the relative concentration of solute and solvent, and we may now turn our attention more particularly to the solute itself. We derived a summation velocity value of 1827 for the hydrated K^+ and Cl^- ions, and in a similar way table II supplies us with corresponding values for other electrolytes. For example:

$Li^o = 6$, $Li^{[+]} = 8$	hydrates with 19 water molecules	$Li^{[+]h} = 222$, $Li^{[+]hv} = 535$
$Na^o = 22$, $Na^{[+]} = 24$	hydrates with 11 water molecules	$Na^{[+]h} = 350$, $Na^{[+]hv} = 671$
$F^o = 18$, $F^{[-]} = 16$	hydrates with 15 water molecules	$F^{[-]h} = 286$, $F^{[-]hv} = 591$

$Li^{[+]hv} = 535$	$Na^{[+]hv} = 671$	$K^{[+]hv} = 1031$
$Cl^{[-]hv} = 796$	$F^{[-]hv} = 591$	$F^{[-]hv} = 591$
Total $= 1331$	Total $= 1262$	Total $= 1622$

With these totals or summation values we may now venture to predict the specific molecular electrical conductivities of any of the given electrolytes from an observed value, temperature and concentration being understood as constant, through solving the equation

$$1827 : 1262 : : 145^2 : x$$

KCl : NaF : : KCl : NaF, etc.

$x = 100.158$. Observed value $= 99.75$.[2]

Using this method it will be found that the observed values for all salts giving rise to monovalent ions of the lighter elements give the predicted conductivities (**11**). If suitable allowances are made for differences in concentration and for the relationship between velocity and conductivity in polyvalent ions, electrolytes comprising the ions Mg^{++}, Ca^{++}, and Al^{+++} may be added to the list. It thus becomes evident that the inverse integral hydration is further substantiated by dynamic phases of solution phenomena beyond the basic values responsible for its development.

[2] Observed values at 0.001 mol. (new weights) and 25° C. by writer.

We may now consider the dynamic phases of the H^+ and OH^- ions, observed as exceptional in that they gave no evidence of being hydrated. From table II we may derive the following velocity values:

$$K^{[+]hv} = 1031$$
$$H^{[+]v} = 5000$$
$$OH^{[-]v} = 2357 \text{ (for 18)}$$
$$= 2500 \text{ (for 16)}$$

We may then venture to predict relative ion conductance at 18° C. as follows, 64.5 being the observed value for the K^+ ion (**10**):

(1) $K^{[+]hv} : H^{[+]v} : : K^{[+]hv} : H^{[+]v}$

$1031 : 5000 : : 64.5 : x \qquad x = 313$

The observed ion conductance of the H ion at 18° C. is 313 (**10**), and the agreement substantiates the other evidence that the H^+ ion is characteristically unhydrated.

(2) $K^{[+]hv} : OH^{[-]v} : : K^{[+]hv} : OH^{[-]v}$

For 18, $1031 : 2357 : : 64.5 : x \qquad x = 148.5$

For 16, $1031 : 2500 : : 64.5 : x \qquad x = 156.5$

The observed ion conductance of the OH^- ion at 18° C. is 174 (**10**). The agreement is not satisfactory, and yet is sufficient clearly to indicate that the OH^- ion is not hydrated, for in the latter case the ion conductance would be of the order of 37. Granting the fact of an exception in this instance, we may tentatively consider 16 to be the expected anhydrous weight of the OH^- ion and proceed with the value 2500 as representing its velocity referred to the base KCl = 1827.

It now becomes of interest to compare the relative velocities of individual ion components of various electrolytes in accordance with the foregoing considerations, and in such a comparison to search for a means of expressing relative velocities in such a way as possibly to serve as an index of the solution characteristic designated as acidity or alkalinity. If we obtain the ratio of the velocity of the faster moving ion to that of the slower ion by simple division, and prefix this by the sign of the faster ion, we have a system of evaluation in which equal velocities are represented as 1.0. Subtracting 1.0 from this value we then have a system in which equal velocities are represented by zero. Such a base would then correspond to neutrality in case acidity and alkalinity were associated with differential activities of the respective positive and negative ions. As examples we have

$H^{[+]v} = 5000$ $Cl^{[-]hv} = 796$ $5000 \div 796 = 6.28$ $6.28 - 1 = 5.28$; ion of greater velocity is positive. Acid-alkali index for HCl = + 5.28 (acid). $Li^{[+]hv} = 535$ $OH^{[-]v} = 2500$ $2500 \div 535 = 4.67$ $4.67 - 1 = 3.67$; ion of greater velocity is negative. Acid-alkali index for LiOH = − 3.67 (alkaline).

In extending this study to include various other electrolytes, it soon becomes apparent that in the polyvalent ions the number of charges is a factor

in the acid-alkali activity. At this point we may grant another exception and tentatively multiply the velocity values by the numbers representing the valence of the ions. The velocity-charge products thus obtained may now be treated as were the simple velocity values to derive indices of acid-alkali activity. In order to comprehend a truly representative series of electrolytes over the acid-alkali range, however, it becomes necessary to extend the inverse integral hydration system, noted in table II as characterizing the weight range of the elements of the first quarter of the periodic system. This hydration system as thus extended and previously published (**12**) becomes periodic with four phases, thus differing somewhat from the periodicity noted by BREDIG (**6**); and it is important to note that under conditions in which ions hydrate, such hydration appears to be a simple function of weight, independent of the nature of the ion. It thus develops that hydrating radicals of a weight comprised within the weight range of the elements may be treated as element ions of the same weight. In so far, therefore, as we are able to classify ions into the respective groups hydrated and unhydrated, we may now venture to predict, from the index obtained through the use of velocity-charge products, the relative acid-alkali reactivity of a solute electrolyte. In table III a number of electrolytes representing the acid-alkali range have been brought together to permit a comparison of their relative positions on the basis of the velocity-charge product index and on the conventional basis designated as pH.

In table III it will be observed that the order of acidity-alkalinity of the electrolytes as derived from a consideration of relative velocity-charge products corresponds with that derived from conventional measurements, except as regards sodium hydroxide, where a partial breakdown of the OH^- ion is suggested. In the conventional measurements, however, the degree of acidity-alkalinity is commonly interpreted as representing the actual presence of a certain concentration of H^+ and OH^- ions. The differences in solutions with respect to their behavior toward indicators or electrodes may well be correlated with certain concentrations of H^+ and OH^- ions *when these ions and these alone are present.* A comparison of the values in columns 7 and 8 of table III, however, leads to the suggestion that in aqueous solutions the velocity-charge products of the solute ions may be responsible for the activity conveniently attributed to specific H^+ and OH^- ion concentrations. The foregoing suggestion does not appear to be at variance with the recognized dominance of the H^+ ion in acidity. For example, we may note the velocity-charge products in some representative hydrogen-replacement series as brought together in table IV.

In table IV it will be noted that replacement of hydrogen uniformly reduces acidity as represented by the velocity-charge product and that alkalinity may result from such replacement.

TABLE III

COMPARISON OF TENTATIVE ACIDITY-ALKALINITY INDEX DERIVED FROM VELOCITY-CHARGE PRODUCTS WITH RELATIVE VALUES OF pH AS CONVENTIONALLY MEASURED

ELECTROLYTE	ASSUMED IONIZATION AND HYDRATION	CALCULATED IONIC WEIGHTS	CALCULATED IONIC VELOCITIES	CALCULATED VELOCITY-CHARGE PRODUCTS		TENTATIVE ACIDITY-ALKALINITY INDEX	OBSERVED* pH IN N/10 SOLUTION
				+ Ion	+ Ion		
HCl	$H^{[+]}$ $Cl^{[-]h}$	$H^{[+]} = 4$ $Cl^{[-]h} = 158$	$H^{[+]} = 5000$ $Cl^{[-]h} = 796$	5000	796	+5.280	1.0
H_2CO_3	$H^{[+]}\ H^{[+]}$ $CO_3^{[--]h}$	$H^{[+]} = 4$ $CO_3^{[--]h} = 380$	$H^{[+]} = 5000$ $CO_3^{[--]h} = 513$	5000	1026	+3.87	3.8
H_3BO_3	$H^{[+]}\ H^{[+]}\ H^{[+]}$ $BO_3^{[-3]h}$	$H^{[+]} = 4$ $BO_3^{[-3]h} = 412$	$H^{[+]} = 5000$ $BO_3^{[-3]h} = 494$	5000	1482	+2.375	5.2
$NaHCO_3$	$Na^{[+]h}$ $HCO_3^{[-]}$	$Na^{[+]h} = 222$ $HCO_3^{[-]} = 60$	$Na^{[+]h} = 671$ $HCO_3^{[-]} = 1290$	671	1290	−0.923	8.4
Na_2CO_3	$Na^{[+]h}$ $CO_3^{[--]}$	$Na^{[+]h} = 222$ $CO_3^{[--]} = 56$	$Na^{[+]h} = 671$ $CO_3^{[--]} = 1336$	671	2672	−2.98	11.6
NaOH	$Na^{[+]h}$ $OH^{[-]}$	$Na^{[+]h} = 222$ $OH^{[-]} = 16$	$Na^{[+]h} = 671$ $OH^{[-]} = 2500$	671	2500	−2.72	13.1
NaOH	$Na^{[+]h}$ $Na^{[+]h}$ $O^{[--]}$	$Na^{[+]h} = 222$ $H^{[+]} = 382$ $O^{[--]} = 12$	$Na^{[+]h} = 671$ $H^{[+]h} = 512$ $O^{[--]} = 2887$	591.5 (average)	5774	−8.76	13.1

* The ABC of hydrogen ion control. LaMotte Chemical Products Co., Baltimore, Md. 1926. p. 15.

TABLE IV

COMPARISON OF CALCULATED AND OBSERVED VALUES FOR ACIDITY AND ALKALINITY IN AQUEOUS SOLUTIONS OF CERTAIN ELECTROLYTES

ELECTROLYTE	ASSUMED IONIZATION AND HYDRATION	CALCULATED IONIC WEIGHTS	CALCULATED IONIC VELOCITIES	CALCULATED VELOCITY-CHARGE PRODUCTS		TENTATIVE ACIDITY-ALKALINITY INDEX	OBSERVED (7) RELATIVE ACIDITY-ALKALINITY REACTION
				+ ION	− ION		
H_3PO_4	$H^{[+]}$	4	5000	5000	460	+9.87	Strong acid
	$H_2PO_4^{[-]h}$	474	460				
$H_2PO_4^-$	$H^{[+]}$	4	5000	5000	890	+4.62	Acid
	$HPO_4^{[--]h}$	506	445				
HPO_4^{--}	$H^{[+]}$	4	5000	5000	2697	+0.855	Weak acid
	$PO_4^{[-3]h}$	124	899				
NaH_2PO_4	$Na^{[+]h}$	222	671	671	460	+0.458	Extremely weak acid
	$H_2PO_4^{[-]h}$	474	460				
Na_2HPO_4	$2\,Na^{[+]h}$	222	671	671	890	−0.326	Weak alkali
	$HPO_4^{[--]h}$	506	445				
Na_3PO_4	$3\,Na^{[+]h}$	222	671	671	2697	−3.02	Alkali
	$Po_4^{[-3]h}$	124	899				

On the other hand an analogous study of alkalinity does not lead to the suggestion that the hydroxyl ion, OH^-, has a correspondingly dominant rôle in alkalinity; but rather that the oxygen ion, O^{--}, is the most active of the common negative ions. This suggestion was evidenced by the NaOH relationships in table III. Through analogy between sulphur and oxygen, further comparisons may be made as in table V.

An examination of table V with reference to alkalinity shows the following velocity-charge products for H_2O in the ionization assumed:

$$H_2O \text{ as } H^+,\ OH^- = +1.00$$
$$OH^- \text{ as } H^+,\ O^{--} = -0.155$$

These values suggest that with the assumed breakdown of the OH^- ion an amphoteric activity of the range $+1.00$ to -0.155 may be ascribed to unhydrated H^+, OH^-, and O^{--} ions in water, on which basis it would appear that the reaction of water should theoretically be slightly acidic rather than neutral.

Following up this suggestion it is of interest to go back to our observed ion conductance value for the OH^- ion at 18° C., 174. If there is the breakdown of the OH^- ion assumed as accountable for the amphoteric activity of water, the velocity-charge products represented would be $OH^- = 2500$ and $O^{--} = 5774$. Per unit electrical charge, the average value would thus be 2758. If we now use this value instead of the value $OH^- = 2500$ to predict the ion conductance of the so-called OH, we have

$$K^{[+]hv} : OH^{[-]v} : : K^{[+]hv} : OH^{[-]v}$$
$$1031 : 2758 : : 64.5 : x$$

Solving, we obtain 172.5, a value in substantial agreement with the observed value 174. It is fully realized that these and the foregoing considerations with respect to the reactivity of the ions of water are in the nature of tentative assumptions; and yet it is obvious that they have an intimate association with observed facts through the medium of amphoteric activity, ion conductance, and normally slight preponderance of "acidity." The conventional interpretation of pH does not have any obvious association with these facts and practically overrides experimental data in assigning a normal neutrality to water. It further becomes obvious that many electrolytes giving rise to none but hydrated ions in aqueous solutions would have an acid-alkali activity falling within the foregoing amphoteric activity of water, and would thus *appear* to be "neutral" salts. The "neutrality" of these salts has long been recognized as only relative, however, while their differing actions as to the modification of pH readings is also familiar. The rôle of buffers in the new description of hydration as related to acid-alkali reactivity is most intriguing, but cannot be taken up at this time.

Generalizing from the foregoing comparisons, it appears that when aqueous solutions of electrolytes are characterized by hydrated ions their

TABLE V

COMPARISON OF CALCULATED AND OBSERVED ACIDITY-ALKALINITY VALUES FOR CERTAIN ELECTROLYTES

Series	Electrolyte	Assumed ionization and hydration	Calculated ionic weights	Calculated ionic velocities	Calculated velocity-charge products		Tentative acidity-alkalinity index	Observed (10) reaction
					+ Ion	− Ion		
Sulphur	H_2S	$H^{[+]}$	4	5000	5000	1826	+1.74	Weakly acidic
		$HS^{[-]}$	30	1826				
	HS-	$H^{[+]}$	4	5000	5000	3780	+0.32	Very weakly acidic
		$S^{[--]}$	28	1890				
	NaHS	$Na^{[+]h}$	222	671	671	1826	−1.72	Weakly alkaline
		$HS^{[-]}$	30	1826				
	NaS-	$Na^{[+]h}$	222	671	671	3780	−4.64	Alkaline
		$S^{[--]}$	28	1890				
Oxygen	H_2O	$H^{[+]}$	4	5000	5000	2500	+1.00	Assumed as neutral, but generally observed as slightly acidic
		$OH^{[-]}$	16	2500				
	OH-	$H^{[+]}$	4	5000	5000	5774	−0.155	
		$O^{[--]}$	12	2887				
	NaOH	$Na^{[+]h}$	222	671	671	2500	−2.72	Alkaline
		$OH^{[-]}$	16	2500				
	NaO-	$Na^{[+]h}$	222	671	671	5774	−7.60	Strongly alkaline
		$O^{[--]}$	12	2887				

reaction tends to be relatively neutral; that when the positive ions are not hydrated their reaction tends to be acidic; and that when the negative ions are not hydrated their reaction tends to be alkaline. As yet we have no information concerning the factors which condition hydration; yet the validity of an assumed state with respect to hydration may be checked by measurements of diverse phases of solution phenomena, as has been shown. When these measurements yield a consistent interpretation it would appear that the velocity-charge product may render a real service in the evaluation of the acid-alkali characteristic of solutions of electrolytes.

For some years now we have been measuring pH in all sorts of situations, in tissue fluids, in streams, in soils, etc., and have accumulated considerable literature as a result. Yet who among us feels that we are getting anywhere in this research? In one locality a certain legume does not thrive in a soil of a certain pH and we conclude that the soil is too acid; yet in another locality the plant may be thriving in a soil with a higher acidity. The literature includes many such examples of apparent inconsistency accompanying the interpretation of pH measurements involving unknowns. In other words, physiological research has amply demonstrated that, with relation to organisms at least, the pH requirement is not a constant thing.

Nor is physico-chemical research without evidence to this same end. Notwithstanding the alleged specificity of the hydrogen electrode, there appear to be substantial reasons for associating acid-alkali reactivity with *all ions present*, and if with all ions present, then variable with the nature of the ions. There is no question but that most solution characteristics (electrical conductivity, freezing point, boiling point, etc.) involve the participation of all ions present; moreover, that these characteristics fail to demonstrate the presence of any such H^+ and OH^- ion concentration as that essential under the conventional interpretation of pH. For example, an aqueous solution of HCl gives an electrical conductivity both representable and predictable as the sum of the ion conductances characterizing the H^+ and Cl^- ions respectively, the individual values being the same in other electrolytes, such as H_2SO_4 and KCl. The observed ion conductance of the OH^- ion is of the order of three times that of the Cl^- ion. Under the conventional interpretation, therefore, the assignment of a pH value to an aqueous solution of HCl requires the assumed presence of OH^- ions under conditions in which they are not demonstrable, and in which they would be expected to become demonstrable if present. We are familiar with the labored conventional explanation of what happens, but it is difficult to overlook the fundamental principle of ionization, an equal number of positive and negative charges. The régime of H^+ ions see-sawing with OH^- ions while all other ions disappeared by request for the time being has been a hey-day of hope, but little real progress in research has been made.

Are we not justified, therefore, in examining this other view-point? It has obvious limitations. It will not directly help one bit in the study of unknown substances, but it may at least enlighten us as to the futility of expecting to accomplish anything with acid-alkali measurements independent of a knowledge of the involved ions. In thus broadening our viewpoint to include all ions, however, we may put ourselves in a position to make worthwhile advances in research.

In conclusion the writer desires to point out with emphasis that *no criticism of the conventional methods of measuring acid-alkali reactivity is intended;* simply that the measurements thus derived may be subject to another interpretation.

Summary

A study of the ionic velocity-charge products of representative solute electrolytes suggests that acidity and alkalinity are solution characteristics accruing from the dominant activity of positive and negative ions respectively, and bear no essential relation to the presence of hydrogen or hydroxyl ions.

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INFLUENCE OF SHORTER LIGHT RAYS UPON ABSORPTION OF NITRATE BY THE YOUNG WHEAT PLANT[1]

W. E. Tottingham, H. L. Stephens, and E. J. Lease

(with two figures)

Introduction

In a previous publication (**11**) evidence was presented that radiations of highest frequency in the visible spectrum and lowest in the ultraviolet region promote absorption of nitrate by the young wheat plant. Those results indicated that yellow to violet rays also increase the synthesis of protein in this plant, while the longer ultraviolet do not. Popp (**9**) had already observed beneficial effects of the shorter visible rays of sunlight in the development of various plant species, but he concluded that these did not modify chemical composition of plant tissues appreciably and that the ultraviolet region is dispensable. Warburg (**12**) found indications of a complementary relation between respiration of carbohydrates and assimilation of nitrate in *Chlorella*. There seems to be lack of substantial demonstration that direct photochemical reduction of nitrate, known to occur *in vitro*, functions extensively *in vivo*.

The present work was planned to deal only with gross fractions of the spectrum, leaving more detailed investigation to those equipped for operation under selected wave lengths of light. Since the regulation of spectral portions of sunlight to equal intensity on a large scale without modifying the distribution of wave length is not readily accomplished, lamps have been used.

Experimentation

Wheat seed[2] was selected from a pure line stock of the Progress variety, this being an awned spring type resembling Marquis. After surface sterilization and soaking it was germinated between papers at about 20° C. When the plumules were emerging, seedlings of uniform appearance were fixed on paraffined wire netting suspended over the nutrient solution in battery jars. The latter were 14.3 cm. in diameter, with a capacity of 3.3 liters, and had been painted black on the exterior to exclude light. During the first day the plants were partially shaded to favor establishing of the root system, and three or four days thereafter the population was reduced to 50 per jar.

[1] Published with permission of the Director of the Wisconsin Agricultural Experiment Station. Supported by annual grants from the Committee on the Effects of Radiation upon Living Organisms, Division of Biology and Agriculture, National Research Council.

[2] Supplied through the kindness of Prof. B. D. Leith, of the Department of Agronomy, this institution.

The nutrient solution had a total concentration of 0.24 per cent., with distribution gram-molecularly as follows: 0.0010 $MgSO_4$, 0.0016 $Ca(H_2PO_4)_2$, 0.0040 NaCl, 0.010 KCl, 0.0090 $NaNO_3$, and about 0.00004 ferric citrate. Most of the salts were of a special lot prepared under specifications for the Committee on Salt Requirement of Plants, National Research Council (**6**). Others were C.P. brands but the NaCl and ferric citrate were of commercial grade. Distilled water was provided as condensate derived from the steam heating service of the greenhouse. This provided a solution which favored elongation and production of dry matter by the plants as much as did one prepared by redistilling the water in glass.

Following complete weekly renewal of the nutrient solution, the plants were transferred to chambers for climatic control 114 days after placing in the culture jars. A few series, as indicated by the data, had been raised under Mazda lamps but at the usual temperature range of 17°–20° C. For those grown in the greenhouse, average intensities of light for the effective length of day have been computed from records of the local station of the U. S. Weather Bureau. Use was here made of the proportion 1 hr. cal. = 140 f.c., as derived earlier (**10**).

The chambers employed for climatic control had been modified since their earlier description (TOTTINGHAM **10**), as shown in figure 1.[3] Use of the humidostat was discontinued, owing to occasional sticking and overheating of the damper-closing solenoids. In lieu of this service the damper between the blower and humidifying chamber was adjusted manually. A value of about 65 per cent. relative humidity of the atmosphere was adopted as favorable to the plants at the temperature effective (14.5°–16.5° C.). Difficulties during earlier tests had led to the adoption of artificial illumination exclusively. The intake of the humidifying fan was so connected as to draw outdoor air through the lamp dome above the culture chamber, this serving to supplement use of the water cells in reducing the heating effects of illumination. It also renewed the supply of CO_2 for the plants. While the degree of control over temperature and humidity was less satisfactory than that recorded earlier (**10**), it was possible to establish somewhat compensatory variations.

Four reflectored 500-watt Mazda C lamps suspended above the water cell in each chamber were operated for a period of 16 hours daily. Supplementary short wave radiation was provided in one chamber by use of a Thomsen arc lamp operating uninclosed at 7.5 amp. on 110 V. The rays were reflected horizontally by a vertical, parabolic mirror through common glass in the door of the chamber, thus screening out injurious shorter ultraviolet rays. The lamp carbons were 13 mm. in diameter, one being solid

[3] We are indebted to the General Electric Company for providing the double-contact magnetic switches used in connection with the heating units.

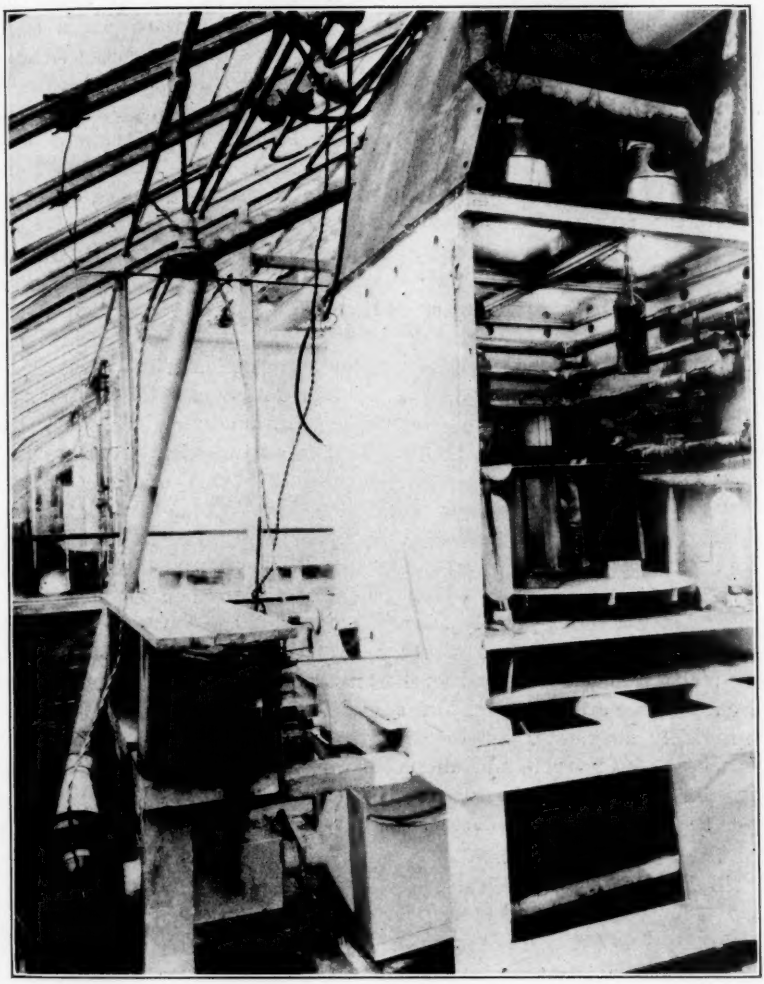

FIG. 1. Climatic chamber, with attachments: The hygrothermograph is riding on the elevated rotating table, as used for young plants. Hanging at the front is the thermostat, which operates luminous radiator units against the refrigerating coils. Note the ventilated lamp dome above the water cell and the humidifying chamber beneath the supporting table. The magnetic switch for heating current hangs on the back of the chamber and the voltage transformer for use with the thermostat is partly visible at the base of the chamber on the left.

and the other cored.[4] An equivalent increase in total radiation in the control chamber was provided by similar use of a 150-watt Mazda lamp. These adjustments, together with measurements of total intensity of light, were made by use of a thermopile receiver and suitable glavanometer.[5] Foot candle equivalents of the radiation were derived by comparison with a new 150-watt Mazda lamp and found to be 1600 f.c. in the starvation period, with 80 f.c. additional during use of the arc lamp, these values being taken as effective at the uppermost plane of horizontally disposed leaves.

For evaluating the general effect of supplementary use of the carbon arc upon the distribution of energy, comparative measurements[6] were made upon this lamp and the ordinary Mazda lamp. Screens of special glasses[7] were used for this purpose in conjunction with a water cell to absorb heat. One of these (Noviol A) transmitted essentially radiations greater than 4200 Å, thus eliminating ultraviolet and a large portion of the violet region of the spectrum. The other (G34 Y) transmitted essentially radiations longer than about 6000 Å, thus eliminating ultraviolet and shorter visible radiations up to the orange region. Results obtained in this manner are shown in table I, the values having been taken at the same distance (45 cm.) as was effective with the plants. These data show that the effective increase of ultraviolet and violet radiation by supplementary use of the carbon arc was only about 1.0 per cent. of the total radiation, or less than 4 per cent. of the spectral region in question. This is much less than our earlier estimate of increase (**11,** p. 2443), in which too high value was accredited an arc lamp of low amperage. There was a somewhat smaller increment in the total region of the radiations shorter than orange. Our screens fail to give a separate evaluation for blue and violet rays, which to the eye appear appreciably augmented by use of the arc. While recognizing that frequencies both favorable and unfavorable to absorption of nitrate may be included within any of the spectral regions here defined, we have proceeded upon the basis that a net effect of such a system should be determinable.

Tests with single salts

When transferred to the control chambers, the cultures were supplied with a nitrate-free solution formed by omitting this salt from the usual

[4] Appreciation is expressed for a gift of carbons from the National Carbon Company.

[5] The thermopile was supplied by the Eppley Company and the galvanometer by the Leeds Northrop Company.

[6] For advice and aid in these measurements we are indebted to Royce E. Johnson, director of the Electrical Standards Laboratory of this institution.

[7] Supplied by the Corning Glass Works. The transmissions of these glasses are given in Technologic Paper 148, U. S. Bureau Standards, 1920. Noviol A is covered by curve 23, fig. 7 and G34 Y by curve 11 × 26, fig. 23. Our plates were 4 mm. thick, however, and would therefore vary somewhat from the transmissions quoted.

TABLE I

INTENSITY AND DISTRIBUTION OF LIGHT EMITTED FROM THE MAZDA LAMP AND CARBON ARC

LIGHT SOURCE	SPECTRAL REGIONS						DISTRIBUTION OF TOTAL LIGHT IN REGIONS OF SHORTER RADIATIONS	
	3100 TO 7600 Å		4200 TO 7600 Å		6000 TO 7600 Å			
	GALVANOMETER DEFLECTION	PROPORTION OF TOTAL LIGHT	GALVANOMETER DEFLECTION	PROPORTION OF TOTAL LIGHT	GALVANOMETER DEFLECTION	PROPORTION OF TOTAL LIGHT	SHORTER THAN BLUE	SHORTER THAN ORANGE
	cm.	%	*cm.*	%	*cm.*	%	%	%
110 V. a.c., 500-watt Mazda lamp	52.9	100	38.4	73	33.8	64	27	36
110 V. a.c., 7.5 amp.* carbon arc	18.9	100	11.0	58	10.1	53	42	47
4 Mazda lamps supplemented by carbon arc†	162.5	100	117.4	72	103.4	64	28	36

* Average of 5 maximum and 5 minimum readings.

† Computed on the basis of a 20 per cent. time relation of the arc to the Mazda lamps, and factor of three for average intensity of four Mazda lamps as placed.

solution. This medium was supplied for a period of four days, during which time the plants were allowed to become adjusted to the new climatic environment. A preliminary test had demonstrated that all nitrate disappeared from plants starved under similar conditions. For an additional final period of three days the plants were subjected to a supply of nitrate only. This practice appeared to be justified by the results of GERICKE (3), who found wheat well nourished by rotation of the usual nutrient salts one by one in intervals of a few days each. It was assumed that this procedure would minimize the influences of other ions upon absorption of NO_3. In order to avoid abrupt changes in osmotic relations between solution and plant, the single nitrates were supplied in concentrations approximating that of the complete nutrient solution. Thus, the solution of KNO_3 contained 0.225 per cent. salt, equivalent to 0.31 mg. N per cc., and the other nitrates were used in equi-normal concentrations.

At the end of the test period the roots were rinsed into the residual solution and the latter was restored to its original volume, for the determination of NO_3. Aliquots of the unplanted nutrient solution were similarly analyzed to provide a base line for the expression of absorption values. The nitrate determination was carried out by the colorimetric procedure of HARPER (5). To provide a basis for relating absorption rates to the organism, the weight of the entire plants in each culture was determined after drying at 98° C. In most cases the effect of a given treatment represents the average of two or more cultures.

In two tests comparison was made of the nitrate carriers under the combined lamps only. These gave unusually high absorption from KNO_3 (53 and 61 mg. N per culture), and in each case 55 per cent. as great absorption from $NaNO_3$. One of these trials included $Ca(NO_3)_2$, with an absorption only 16 per cent. of that from KNO_3. Calcium was included in seven other tests and magnesium in two. These carriers proved relatively inefficient, giving no absorption of nitrate in some cases. No consistent relation of these effects to either the plane of preparatory illumination or the temperature of the test was apparent. A record of those tests in which the responses with sodium and potassium nitrates were compared under the two types of radiation is presented in table II. Dates are included to serve as indices of the character of solar radiation during the preparatory growth period.

From these data (table II) it appears that the range of nitrate absorption per unit of tissue was rather wide, namely, 15 to 31 mg. In this respect the function was independent of both the plane of preparatory irradiation and the temperature of the test. If one considers arbitrarily that only departures of 10 per cent. and more in absorption are significant, it appears that the relatively small increase of shorter radiations involved

TABLE II

RADIATION AND CARRIER EFFECTS IN ABSORPTION OF NITRATE BY YOUNG WHEAT PLANTS; VOLUME OF NUTRIENT SOLUTION 3000 CC. PLANTS SUBJECTED TO PREPARATORY NITRATE STARVATION. TEMPERATURE OF CLIMATIC CHAMBERS 15.5° ± 1.0° C. UNLESS SPECIFIED OTHERWISE, AND RELATIVE HUMIDITY ABOUT 60 PER CENT. INCREASE OF VIOLET TO ULTRAVIOLET INCLUSIVE BY USE OF THE CARBON ARC 1.0 PER CENT.

NUMBER OF TEST	DATE OF COMPLETION	PLANE OF PREPARATORY SOLAR RADIATION	ABSORPTION OF N PER GM. DRY TISSUE FROM KNO_3 UNDER COMBINED LAMPS	RELATIVE ABSORPTION BASED ON METAL CARRIER. PROPORTION WITH USE OF $NaNO_3$ AS RELATED TO KNO_3		RELATIVE ABSORPTION BASED ON SOURCE OF RADIATION. PROPORTION WITH USE OF MAZDA LAMP AS RELATED TO COMBINED LAMPS	
				UNDER MAZDA	MAZDA + ARC	WITH $NaNO_3$	KNO_3
			mg.	%	%	%	%
1	3/23/31	1200*	25	7	39	9	50
4	12/5/30†	1900	22	96	88	80	73
8	2/9/31	2540	17	71	122	74	127
9	3/11/30	2720	24	17	109	13	84
11	3/9/31	2420	17	94	80	104	90
13	4/7/30	3740	25	139	72	176	92
14	3/17/31	3920	31	102	93	91	83
15	4/22/30	4540	15	80	77	198	191
16	5/5/31†	4800	27	80	57	66	47

* Irradiated by Mazda lamp.

† Test conducted at 21.0° ± 1.0° C.

here promoted nitrate absorption. Under these conditions potassium excelled sodium as a carrier of this radical or ion. The beneficial effects upon root condition of both irradiation by the arc lamp and supplying potassium are shown in figure 2.

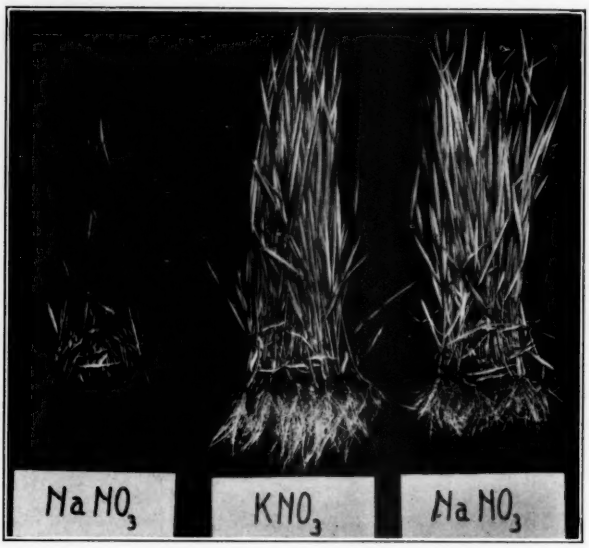

FIG. 2. Showing benefits upon root development from the increase of shorter light rays and the selection of nitrate carrier: Plants at left supplied $NaNO_3$ under the Mazda lamp only; plants at right supplied $NaNO_3$ under the combined Mazda and arc lamps; plants in center supplied KNO_3 under the combined lamps.

A summary of the results when $NaNO_3$ and KNO_3 were directly compared is presented in table III. Here are assembled the number of repetitions (in a total of eleven tests on the usual volume of nutrient solution) of departures exceeding both 5 and 10 per cent. in the absorption of nitrate. In comparing the efficiency of the radiation or carrier on the basis of a 5 per cent. departure, values between 95 and 105 per cent. have been considered as equal. Similarly, for the 10 per cent. departure values between 90 and 110 per cent. have been rated as equal. From these comparative data it appears that potassium excelled sodium as a carrier of nitrate. If the results of tests 3 and 10, which involved only the use of the combined lamps, are omitted, the superiority of potassium over sodium was essentially equal under the two forms of radiation. It is apparent also that supplementary use of the carbon arc favored the absorption of nitrate from both carriers. This superiority of potassium is in agreement

TABLE III

SUMMARY OF DEPARTURES IN ABSORPTION OF NITRATE AS RELATED TO BOTH CHARACTER OF RADIATION AND KIND OF METAL CARRIER. NINE TESTS UNDER COMBINED LAMPS AND MAZDA LAMP ALONE, TWO OTHERS UNDER COMBINED LAMPS ONLY

PERCENTAGE EXTENT OF DEPARTURE	COMPARISON OF SOURCES OF RADIATION						COMPARISON OF METAL CARRIERS					
	SUPPLYING KNO_3			SUPPLYING $NaNO_3$			UNDER MAZDA LAMP AND CARBON ARC			UNDER MAZDA LAMP ALONE		
	COMBINED LAMPS SUPERIOR	RADIATION EFFECTS EQUAL	MAZDA LAMP ALONE SUPERIOR	COMBINED LAMPS SUPERIOR	RADIATION EFFECTS EQUAL	MAZDA LAMP ALONE SUPERIOR	KNO_3 SUPERIOR TO $NaNO_3$	CARRIER EFFECTS EQUAL	$NaNO_3$ SUPERIOR TO KNO_3	KNO_3 SUPERIOR TO $NaNO_3$	CARRIER EFFECTS EQUAL	$NaNO_3$ SUPERIOR TO KNO_3
5 and above	7	0	2	6	1	2	9	0	2	6	2	1
10 and above	6	1	2	5	2	2	8	2	1	6	2	1

with the conclusion of GERICKE (**3**) that KNO_3 excels other nitrates when supplied to wheat in a rotation of single salts. The results suggest that increased absorption might be expected to follow raising the proportion of shorter radiations above the relatively low level of these tests.

In this connection it is of interest to note that COOPER (**2**) gives the energy of formation of KCl as equivalent to ultraviolet light, and suggests that an equivalent of radiant energy may be necessary for its optimal assimilation. This might imply a favorable relation of shorter rays of light to decomposition and assimilation of KNO_3. However, he places infra-red as adequate for the reduction of nitrate. NEMEC (**8**) found the general regions about both violet and red of the spectrum favorable to the absorption of potassium by the rye plant.

TESTS OF LIMITING FACTORS

As this investigation progressed certain tests were applied to contribute toward the elimination of limiting factors.

It was found that plants raised in the greenhouse in April to May removed per unit of dry matter about twice as much NO_3 from the complete nutrient solution at three weeks of age as compared with that removed at six weeks. The effect per unit plant was 70 per cent. as great at the earlier stage. These results indicate that the age of the plant here employed was a favorable factor.

A second test related to high metabolic requirement of potassium as possibly determinative of superiority of this element as a carrier of NO_3. Doubling the supply of KCl during the nitrate starvation period did not affect the plane of subsequent absorption from KNO_3 but depressed the intake from $NaNO_3$.

A third test was concerned with the relation of solution volume to nitrate absorption. This was actuated by the assumption that more definite evidence might follow a wider differential of NO_3 content between planted and unplanted solutions if the latter were supplied in smaller as compared with larger volumes. Approximately one-eighth the usual volume of solution was supplied in shallow dishes of the same diameter as the larger jars. The evidence supported that already presented, for in two of three trials KNO_3 exceeded $NaNO_3$ in uptake, and was absorbed to a greater extent under the combined forms of radiation than under the Mazda lamp alone. However, the plane of absorption (6 to 11 mg. N per culture) was unusually low. Stirring by slow aeration increased the absorption of KNO_3 18 per cent. and of $NaNO_3$ 12 per cent. in a greenhouse test under ordinary illumination, about one-third of the supply being removed from the stagnant solutions. In view of the free surface exposure and shallowness of these solution portions, it appears possible that the rate of renewal of salt

at the root surface was more potent than increased oxygen supply in stimulating absorption. Moreover, it should be noted that ALLISON and SHIVE[8] found aeration favorable to tissue production by soy beans only as the nutrient solution was continually renewed; yet increased aerobic respiration should be admitted as a possible favorable factor here. We purpose giving attention to this issue.

TESTS WITH COMPLETE NUTRIENT SOLUTION

The irregularities of response noted in the preceding work indicated the desirability of seeking other limiting factors. This search was directed to the use of the complete nutrient solution as well as to those of single nitrates. It included a test of omission of the nitrate starvation period, and modification of the arc lamp for the purpose of increasing the proportion of ultraviolet radiation.

Sodium nitrate formed about one-third of the complete salt mixture by weight, so that the nitrate concentration in the complete nutrient solution

TABLE IV

INFLUENCE OF PRELIMINARY STARVATION UPON SUBSEQUENT NITRATE ABSORPTION FROM A COMPLETE NUTRIENT SOLUTION BY YOUNG WHEAT PLANTS. PREPARATORY GROWTH UNDER 1200 F. C. FROM THE MAZDA LAMP AT 18°–20° C. TEST AT 15.5° ± 1° C.

PRELIMINARY NUTRIENT TREATMENT	SOURCE OF RADIATION	NITROGEN ABSORBED PER GM. DRY TISSUE PER CULTURE	ABSORPTION IN RELATION TO CHARACTER OF RADIATION	ABSORPTION IN RELATION TO STARVATION PRACTICE
		mg.	%	%
Nitrate starved	Mazda lamp only	12.3		
		7.0		
		10.9		
		11.6		
		Ave. 10.5	100	100
Nitrate starved	Mazda + arc lamp	11.4		
		11.2		
		12.2		
		10.4		
		Ave. 11.3	108	100
Unstarved	Mazda lamp only	10.5		
		10.4		
		8.7		
		11.6		
		Ave. 10.3	100	98
Unstarved	Mazda + arc lamp	18.5		
		16.0		
		16.7		
		18.0		
		Ave. 17.3	168	153

[8] Amer. Jour. Bot. **10**: 554–566. 1923.

bore that proportion to the single salt solution. The results of one test are shown in table IV, which includes the individual variability of cultures. It appears that the increase in radiation of shorter wave length was decidedly beneficial to the unstarved plants but only questionably so to those pre-starved.

Other tests were conducted under higher planes of preparatory illumination, as indicated in part by the date of completion, but with the usual plane of 1600–1680 f.c. illumination obtaining in the chamber. Unstarved plants reared on 4100 f.c. average illumination and finished on April 26 gave 20 per cent. greater nitrate absorption by supplementary use of the arc lamp. A subsequent test was completed May 15 at about 21° C. after preparatory irradiation at 4800 f.c. This gave increased absorption under supplementary use of the arc of 19 per cent. on the complete solution, 111 per cent. on KNO_3, and 51 per cent. on $NaNO_3$. In this case the absorption of nitrate per unit of dry matter from the complete solution declined appreciably (from 11–15 to 8–9 mg. N) at the higher temperature. This response may be related to the accumulation of nitrate observed earlier (**10**, table IX) at high temperature, which would tend to limit the absorption process. The higher attendant respiratory activity could be expected to decrease the plane of reducing substances in the tissue, and thus to limit both assimilation and absorption of NO_3. A test completed November 30 under 1300 f.c. average preparatory solar radiation gave an increased nitrate absorption of 25 per cent. from the complete solution under the com-

TABLE V

INFLUENCE OF PRELIMINARY NITRATE STARVATION UPON NITRATE ABSORPTION FROM A SOLUTION OF KNO_3 BY YOUNG WHEAT PLANT. PREPARATORY GROWTH UNDER 2000 F.C. RADIATION FROM THE MAZDA LAMP

SOURCE OF RADIATION	PRELIMINARY NUTRIENT TREATMENT	WEIGHT OF ENTIRE DRIED PLANT	NITROGEN ABSORBED PER GM. DRY TISSUE	ABSORPTION IN RELATION TO CHARACTER OF RADIATION (AVERAGE)	ABSORPTION IN RELATION TO STARVATION PRACTICE (AVERAGE)
		gm.	*mg.*	%	%
Mazda lamp only	Starved	5.3 5.6	14.0 14.0	100	100
Mazda and arc	Starved	5.7 5.4	14.2 14.6	103	100
Mazda lamp only	Unstarved	5.7 6.0	15.1 15.9	100	111
Mazda and arc	Unstarved	5.9 6.1	18.1 16.2	111	120

bined lamps. The absolute amounts of nitrogen absorbed from $NaNO_3$ and KNO_3 were of the same order as from the complete solution, and increased 18 and 20 per cent. respectively by supplementary use of the carbon arc.

A further test of the influence of nitrate starvation upon subsequent absorption was made with a nitrate alone. The results are given in table V, wherein the weight of dried plants is included to show the extent of variation of this factor. From these data it is further evident that preliminary starvation in respect to NO_3 was an unfavorable feature of our earlier tests. The plane of absorption from a complete solution was also favorable in unstarved plants, as shown in table VI.

TABLE VI

INFLUENCE OF INCREASED PROPORTIONS OF SHORTER RADIATIONS UPON ABSORPTION OF NITRATE BY YOUNG WHEAT PLANTS WITHOUT PRELIMINARY NITRATE STARVATION. AVERAGE PLANE OF SOLAR RADIATION IN PREPARATORY GROWTH PERIOD 1540 F.C. COMPLETED DECEMBER 16, 1931

SOURCE OF RADIATION	FORM OF NITRATE CARRIER	NITROGEN CONTENT OF ORIGINAL SOLUTION PER LITER	WEIGHT OF ENTIRE DRIED PLANTS	NITROGEN ABSORBED PER GM. DRIED TISSUE	ABSORPTION IN RELATION TO RADIATION (AVERAGE)
		mg.	*gm.*	*mg.*	%
Mazda lamp only	Complete solution	122	6.6	19.1	100
		122	6.3	19.5	
	KNO_3	124	5.6	15.4	100
	$NaNO_3$	125	5.9	16.5	100
Mazda and arc	Complete solution	122	5.7	22.2	115
		122	5.5	22.2	
	KNO_3	124	5.6	18.7	121
	$NaNO_3$	125	6.1	18.9	115

IMPROVEMENT OF CARBON ARC

Increased amperage results in an increase of short wave emission by the carbon arc, as shown by COBLENTZ and associates (**1**). With the aid of the Service Department of this institution our lamps were reconstructed to give this effect. A part of the current was shunted around the lamp resistance and the entire unit was operated through an appropriate rheostat. Current consumption was thus converted from a value of 7.5 amperes at 110 volts to 22 amperes at 45 volts, across the arc. The reconstructed lamp was equipped with impregnated carbons of the Sunshine type, 13 mm. in diameter. An examination in the manner already indicated of the intensity and distribution of radiation from the altered lamp included the use of an additional glass screen (G586 A, with transmission coefficients given by

GIBSON and associates **4**, fig. 21, curve 83). This transmits strongly in the longer ultraviolet close to the maximum of emission from the arc, and to a slight extent in the red region. The total intensity of the arc from the modified lamp was about 50 per cent. greater than that of the form used earlier, and the same relation held for the regions defined in table I. In the latter regions, therefore, the radiation effects had not been appreciably increased. However, the emission from the newer form of arc transmitted by screen G586 A, which was essentially solar ultraviolet, was more than twice as intense as that from the older form. With the increased time of operation the reconstructed lamp increased the proportion of this spectral region alone by somewhat less than 1 per cent. of the total radiation emitted by the Mazda lamps, equivalent to an increase of over 5 per cent. in the region specified.

Two series of cultures were conducted with use of the reconstructed arc lamp, the time of differential radiation being two daily periods of six hours each, without preliminary starvation. The data appear in tables VII and VIII, and indicate that the increased proportion of long ultraviolet rays considerably favored the absorption of nitrate. The beneficial effect of a continuous supply of nitrate is evident also in the relatively high planes of weight of nitrogen absorbed.

TABLE VII

INFLUENCE OF IMPROVED CARBON ARC UPON ABSORPTION OF NITRATE BY YOUNG WHEAT PLANTS. AVERAGE PLANE OF SOLAR RADIATION DURING PREPARATORY GROWTH 3410 F.C. TEST COMPLETED APRIL 6, 1932

SOURCE OF RADIATION	FORM OF NITRATE CARRIER	NITRATE CONTENT OF ORIGINAL SOLUTION PER LITER	WEIGHT OF ENTIRE DRIED PLANTS	NITROGEN ABSORBED PER GM. DRIED TISSUE	ABSORPTION IN RELATION TO CHARACTER OF RADIATION
Mazda lamp only	$NaNO_3$	314	6.1	22.1	100
	$NaNO_3$	314	6.0	23.2	
	KNO_3	317	5.4	24.0	100
	KNO_3	317	5.8	23.6	
Mazda + Sunshine carbon arc	$NaNO_3$	314	5.5	28.1	124
	$NaNO_3$	314	5.6	28.2	
	KNO_3	317	5.8	30.1	127
	KNO_3	317	5.6	30.2	

The nearest approach to a comparison of the forms of radiation here applied with sunlight appears to be offered by the data of COBLENTZ and associates (**1**, table II). For the proportion between the region 3500 to 4500 Å and the total emission from 3500 to 6000 Å, these give the following percentages: June sunlight 38, tungsten lamp (115 V. a.c., 12.7 amp.) 21, carbon arc (50 V. a.c., 5 amp.) 53, and white flame arc (70 V. a.c., 20 amp.)

TABLE VIII

INFLUENCE OF IMPROVED CARBON ARC UPON ABSORPTION OF NITRATE BY YOUNG WHEAT PLANTS. AVERAGE PLANE OF SOLAR RADIATION DURING PREPARATORY GROWTH 4050 F.C. TEST COMPLETED MAY 8, 1932

SOURCE OF RADIATION	FORM OF NITRATE CARRIER	NITROGEN CONTENT OF ORIGINAL SOLUTION PER LITER	WEIGHT OF ENTIRE DRIED PLANTS	NITROGEN ABSORBED PER GM. DRIED TISSUE	ABSORPTION IN RELATION TO CHARACTER OF RADIATION (AVERAGE)
		mg.	*gm.*	*mg.*	%
Mazda lamp only	Complete solution	124 124	5.4 5.7	25.1 24.8	100
	KNO_3	322	5.5	21.5	100
	$Ca(NO_3)_2$	311	5.9	23.0	100
	95 mols KNO_3 + 5 mols $Ca(NO_3)_2$	315	5.3	25.0	100
Mazda + Sunshine carbon arc	Complete solution	124 124	6.1 5.5	34.1 33.9	136
	KNO_3	322	5.9	26.1	122
	$Ca(NO_3)_2$	311	6.0	30.0	130
	95 mols KNO_3 and 5 mols $Ca(NO_3)_2$	315	5.7	29.0	116

60. An attempt to apply these values to our experiment gave only approximately 23 per cent. of shorter radiations in our most efficient system. In view of the relative composition of sunlight this is little improvement over exposure to the Mazda lamp only. COBLENTZ and associates (**1**, fig. 18) give a graphic comparison of the general distribution of radiation from the sun and the carbon arc, and LUCKIESCH (**7**) shows the curve of distribution from an ordinary 500-watt Mazda lamp. From these the deficiency of the arc lamp as a means of supplementing the emission of the Mazda lamp in the blueviolet region becomes apparent.

Summary

1. Following preparatory nitrate starvation, Progress wheat plants approaching three weeks of age were exposed to about 1600 f.c. illumination at about 15.5° C. while supplied single nitrates. Under these conditions the NO_3 radicle was absorbed more freely from KNO_3 in the nutrient solution than from the nitrates of other common metals. This absorption was promoted by increases in blue to longer ultraviolet radiations above those emitted by the common Mazda lamp.

2. As here produced and tested, both with single nitrates and with a complete solution, young wheat plants responded to an increase in the proportion of blue to ultraviolet light more decidedly without preliminary

nitrate starvation than when so prepared. With KNO_3 this response was apparently not due to deficiency of potassium in the starved plants.

3. A substantial further increase in the proportion of long ultraviolet radiation promoted the absorption of nitrate somewhat further. Rough measurements of the distribution of radiation here effective indicate that it was decidedly deficient in other shorter radiations as compared with sunlight.

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AMOUNTS OF BOUND AND FREE WATER IN AN ORGANIC COLLOID AT DIFFERENT DEGREES OF HYDRATION[1]

HELEN L. CHRYSLER

(WITH TWO FIGURES)

Introduction

All of the water in a colloidal system does not possess the same physical properties. This is shown by the fact that when the vapor pressure of the water in such systems is reduced, all of the water does not act as a solvent and some of it cannot be removed by pressure or by freezing. The water in the system which is free to exert those properties characteristic of the bulk of the water in dilute solutions has been termed free water in contrast to that part of the water which is bound or held by the colloid.

It is believed that the ability of the cell colloids to bind water has an important significance in certain physiological processes. SHULL (**27**) measured the forces with which the colloidal gels of the seed attract water by putting the seeds in solutions of very high known osmotic concentrations. He found that an air-dry seed of *Xanthium* with 7 per cent. hygroscopic moisture showed an initial imbibition pressure of about 1000 atmospheres.

Cold resistance has been considered by LOTT (**13**), MARTIN (**14**), NEWTON (**19, 20**), ROBINSON (**23**), ROSA (**24**), and others to be related to the amount of hydrophilic colloids present in the tissue fluids. All of these workers believe that the hardier the species the more hydrophilic colloids are present and the greater the imbibition pressure of the tissues. It has been observed that this correlation does not exist when the material is in the unhardened condition. MEYER (**16**), studying cold resistance of the leaves of the pitch pine, obtained results which he regarded as evidence of this same relationship. His later data (**17**), however, seem to show that the binding of water by colloids is not an important factor in the cold resistance of this species.

NEWTON and MARTIN (**22**), in studying the drought resistance of a number of cereals, grasses, and other plants, concluded that the imbibition pressure of the hydrophilic colloids in the plant tissue fluids is the most significant factor in retaining moisture, and that drought resistant varieties can be differentiated from non-resistant varieties by bound water content. FRITSCH and HAINES (**5**) observed that cells of terrestrial algae surviving after a long period of drought have more or less highly viscous protoplasts without any large vacuoles. This was also the normal condition of the more drought resistant species. They consider that this gel condition

[1] Papers from the Department of Botany, the Ohio State University, no. 305.

of the protoplast may indicate that imbibition phenomena are principally concerned in the drought resistance of these plants.

BAILEY and GURJAR (**1**) have investigated the relation of moisture content to respiration in stored wheat. The rate of respiration increased very gradually with increase in the moisture content from 12.5 to 14.78 per cent. Above a moisture content of 14.78 per cent. the rate was greatly accelerated. It has been suggested by GORTNER (**6**) that this sharp break in the respiration curve may indicate that below 14.78 per cent. moisture, all or most of the water was in the bound condition, and that the rapid increase in the respiration rate was related to an increase in the amount of free water present.

WHITCOMB and SHARP (**31**) studied the germination of frozen and unfrozen wheat that had been harvested at different stages of maturity. They found that at a moisture content of 56.2 per cent. or more the seeds were almost invariably killed by freezing at a temperature of –20° to –28° C. for 48 hours. However, when seeds with a moisture content of 50.6 per cent. or less were treated in the same way, there was a great increase in the percentage of germination, the percentage germinating ranging as high as 98 per cent. GORTNER (**6**) suggests that an explanation for this may also be found in the free-bound water equilibrium of the seeds. The data of KIESSELBACH and RATCLIFF (**12**), on the relative germination of seed corn of varying moisture content after exposure to low temperatures, likewise show that death from freezing is directly related to the moisture content of the grain and the temperature of exposure.

These illustrations indicate that the condition of the water in a biological system has a marked effect on the properties of the protoplasm, and give evidence in support of the supposition that bound water is closely related to the physiological processes carried on by living cells.

It was the purpose of this investigation to determine as nearly as possible the amounts of bound and free water in an organic colloid which had been allowed to imbibe various percentages of water while other conditions were kept constant. A study of the relation of the concentration of dry matter in a colloidal system to the free-bound water ratio, over a wide range of hydrations comparable with those found in protoplasm, should give information of some value for comparison with the complex colloidal system in living cells, and may throw some light on the part played by colloids and bound water in certain physiological processes. At the time this work was begun, no studies of this sort had been reported. Recently, however, several investigators (**2, 11, 22**) have published reports of similar studies with other types of colloidal systems. These papers will be referred to in more detail in the discussion.

Methods and material

The material chosen for use in this investigation was the stipe of the Elk kelp, *Pelagophycus porra* (Leman) Setchell. This was selected because it is an excellent example of an organic substance possessing great hydration capacity. The structural components of the apparently non-vacuolate cells of this alga are probably largely carbohydrate gels such as gums and mucilages, which yield both hexoses and pentoses on hydrolysis. These substances are highly hydrophilic.

For the purpose of this investigation, all the kelp stipe was prepared at one time as follows. The sun-dried stipe as received at the laboratory was soaked in tap water until maximum swelling was attained. During this period the water was changed several times each day. Only those pieces that showed the greatest degree of swelling were selected for use in the investigation. These pieces of swollen stipe were in the form of hollow cylindrical tubes 2–3 inches in diameter with walls about 0.25 inch thick. The outer epidermis was scraped off the selected pieces to aid in further washing and to make the pieces as uniform as possible. Next the swollen stipe was cut into pieces approximately 0.5 inch long, 0.25 inch wide, and 0.25 inch in thickness. These pieces were washed in running tap water for 24 hours, thoroughly washed through a number of changes of distilled water, and finally spread out in boxes in the sun to dry. After about three days the material was air-dry. It was then placed in desiccators over fresh calcium chloride to complete the drying process and left until used in the determinations. An analysis of the washed kelp showed the percentage of ash to be about 0.09 per cent.

The free water was determined by the calorimeter or heat-of-fusion method. This method was first introduced by MÜLLER-THURGAU (**18**) and later modified by RUBNER (**25**) and THOENES (**29**). Certain improvements have recently been made in the method by ROBINSON (**23**), ST. JOHN (**28**), SAYRE (**26**), and MEYER (**17**). The equation used in calculating the results of this investigation is also the one developed by these investigators. According to this method the material is cooled to –20° C. and a determination made of the amount of water which is frozen at the end of the cooling period. This method assumes that at –20° C. all of the free water will be converted into ice and that the free-bound water equilibrium is not altered by the freezing. The value for free water is subtracted from the total water and the remainder is considered to be bound water.

The apparatus and procedure used in making the free water, correction factor, and specific heat determinations are in general the same as those described by MEYER (**17**). It will be necessary, therefore, to describe here only the points in which the methods used in this investigation differ from those described in that paper.

In preparing the dried kelp for the determinations, it was taken from the desiccator, weighed, and put in distilled water long enough to reach approximately the desired hydration. It was then taken from the water, wiped off, weighed again, and the percentage hydration calculated. The temperature was kept constant by keeping the beaker containing the kelp and distilled water in a 30° C. oven except when weighings were being made. Preliminary experiments had been made to determine how long it took the dried kelp to imbibe various percentages of water, and how much dry material was needed for determinations at various hydrations, and a working chart prepared from these data.

The hydration percentages used in these determinations ranged from 30 to 1015 per cent. The degree of hydration is expressed as the percentage of water absorbed by the dry kelp. At a hydration percentage of 100, each gram of dry kelp had imbibed one gram of water. In order to make the conditions as uniform as possible for the samples of kelp at the various hydrations, the same time interval was allowed from the time the desiccator-dried kelp was put in distilled water and the time the samples were put in the freezing bath, regardless of the percentage of hydration. This interval was 16 hours, and was based on the time required to reach maximum hydration in preliminary tests.

Water was used as the calorimeter liquid in preliminary tests, but it was soon discovered that heat produced by the imbibition of water by the kelp gave erroneous temperature readings. Benzene was then selected, because it is not appreciably imbibed by the kelp and because its density is low enough so that the material, even when dry, was well immersed in it. Exactly 220 grams of pure benzene with a volume of 250 cc. at 20° C. were used in making each calorimetric determination. A fresh supply of benzene was used for each test.

Fifteen-gram samples of hydrated kelp were used except for the two highest hydrations, when the weight of the sample was reduced to 10 grams. This was necessary because the range of temperature on the calorimeter thermometer was not great enough to measure the lowest temperature of the benzene if 15-gram samples were used.

In all of the determinations reported in this paper, the system was brought to equilibrium by stirring the contents of the calorimeter with a stirring rod made of a loop of iron wire of small diameter. A hole large enough to admit the wire was provided in the stopper of the calorimeter. The time required to reach equilibrium was about 5 minutes.

Calculation of the amount of water which freezes within the material was made according to the following equation. An explanation of the derivation of this equation may be found in the paper by MEYER (**17**).

$$W_f = \frac{FNS(T-T_e) - [W_dS_d(T_e-T_s) + W_wS_w(T_e-T_s)]}{Q - [(S_b-S_l)(T_m-T_s)]}$$

Where

F = Correction factor.
N = Weight of benzene in grams.
S = Mean specific heat of benzene for temperature range $T-T_e$.
T = Temperature of benzene at beginning of test.
T_e = Equilibrium temperature of benzene.
W_d = Weight of dry matter in the sample in grams.
S_d = Mean specific heat of dry kelp for the temperature range T_e-T_s.
T_s = Temperature of the sample at beginning of test.
W_w = Weight of all the water in the sample in grams.
S_w = Mean specific heat of all water in the sample for temperature range T_e-T_m.
S_b = Mean specific heat of unfrozen water for the temperature range T_m-T_s.
T_m = Melting point of ice in the sample = freezing temperature of free water.
S_l = Mean specific heat of ice in the sample for the temperature range T_m-T_s.
Q = Heat of fusion of ice at T_m.

It is necessary to determine a correction factor for the calorimeter, as the heat absorbed by the sample is taken not only from the benzene but also from the walls of the calorimeter and the thermometer and stirrer in contact with the benzene. The value obtained for free water is too low unless this correction is made. The value for the correction factor was taken as 1.05 for a 15-gram sample, this being the average value obtained from twenty closely agreeing determinations. The correction factor for a 10-gram sample would be slightly lower than this. The same value was used, however, as other experiments have shown that the error involved was so small that it could be disregarded without any appreciable effect on the results.

The average value determined for the specific heat of dry kelp was 0.27 calories. This is probably a minimum value, as the recorded values for the specific heat of other substances of similar chemical composition are somewhat higher. The values used for the specific heat of pure benzene were secured by plotting a curve for values as given in the International Critical Tables (**30**). The values for the specific heat of ice and heat of fusion of ice were obtained from tables by DICKINSON and OSBORNE (**3**). The values for the specific heat of water above 0° C. are those given in the Handbook of Physics and Chemistry (**10**). The values for the specific heat of water from 0° to −20° C. were obtained by extrapolating the curve plotted for the values above 0° C. given in the table just mentioned. It has been as-

sumed that the specific heat of bound water is the same as that of free water at the same temperature.

Results and discussion

A series of three calorimetric determinations was made for each hydration percentage. These values and the average values for each hydration are presented in table I. Figure 1 gives curves for the average amounts

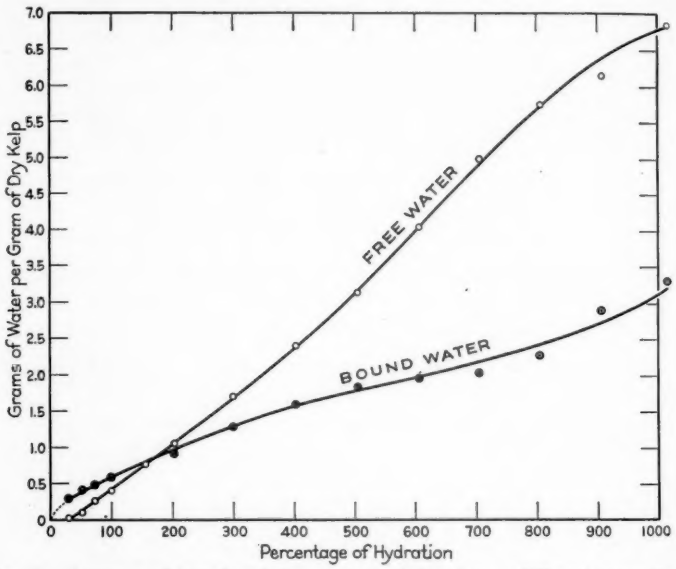

FIG. 1. Amounts of bound and free water in kelp stipe at different percentages of hydration.

of bound and free water per gram of dry kelp at the different percentages of hydration. The amounts of bound and free water per gram of dry kelp are plotted as ordinates against the percentages of hydration as abscissas.

These data show that the amount of imbibed water bound per gram of dry kelp increases with an increase in hydration, but not proportionately; hence the percentage of imbibed water bound decreases with increased hydration. At the lowest hydration of 30 per cent., 0.283 gram of water (95.83 per cent.) was bound per gram of dry material. As the hydration was increased the amounts of both bound and free water per gram of dry material showed consistent increases, the bound water at first increasing more rapidly than the free water, until a hydration of 156.6 per cent. was reached. Here the amounts of bound and free water were approximately equal. Above this hydration the amount of free water per gram of dry

TABLE I

FREE AND BOUND WATER IN KELP STIPE AT DIFFERENT DEGREES OF HYDRATION

HYDRATION PER GM. DRY KELP	DRY KELP	FREE WATER	BOUND WATER	FREE WATER PER GM. DRY KELP	BOUND WATER PER GM. DRY KELP	BOUND WATER AS PERCENTAGE IMBIBED WATER
%	%	*gm.*	*gm.*	*gm.*	*gm.*	%
		0.100	3.312	0.009	0.287	97.07
30.00	76.92	0.095	3.317	0.008	0.287	97.22
		0.232	3.180	0.020	0.276	93.20
Average		0.142	3.270	0.012	0.283	95.83
		0.940	4.021	0.094	0.404	81.05
50.91	66.27	0.851	4.110	0.086	0.415	82.85
		1.043	3.918	0.105	0.394	78.98
Average		0.945	4.016	0.095	0.404	80.96
		2.212	4.064	0.256	0.471	64.75
73.94	57.49	2.157	4.119	0.250	0.478	65.63
		2.005	4.271	0.232	0.495	68.05
Average		2.125	4.151	0.246	0.481	66.14
		3.197	4.253	0.426	0.567	57.09
100.00	50.00	2.932	4.518	0.391	0.602	60.64
		3.098	4.252	0.413	0.567	57.85
Average		3.075	4.341	0.410	0.579	58.53
		4.498	4.597	0.768	0.785	50.54
156.60	39.03	4.449	4.646	0.760	0.794	51.08
		4.563	4.532	0.779	0.774	49.83
Average		4.503	4.592	0.769	0.784	50.48
		5.466	4.334	1.093	0.867	44.22
201.90	33.33	5.527	4.273	1.106	0.855	43.60
		4.822	4.978	0.964	0.996	50.80
Average		5.272	4.528	1.054	0.906	46.20
		6.072	5.078	1.619	1.354	45.54
300.00	25.00	6.382	4.768	1.702	1.271	42.76
		6.589	4.561	1.757	1.216	40.91
Average		6.348	4.802	1.693	1.280	43.07
		7.002	4.959	2.342	1.659	41.46
401.60	19.93	7.447	4.514	2.498	1.510	37.74
		7.198	4.763	2.408	1.593	39.82
Average		7.216	4.745	2.416	1.587	39.67
		7.411	4.912	2.991	1.983	39.86
505.60	16.51	8.249	4.074	3.330	1.645	33.06
		7.625	4.698	3.078	1.897	38.12
Average		7.762	4.561	3.133	1.842	37.01
		8.307	4.516	3.905	2.123	35.22
605.00	14.19	8.186	4.637	3.849	2.180	36.16
		9.431	3.392	4.434	1.595	26.45
Average		8.641	4.182	4.063	1.966	32.61
		9.183	3.952	4.925	2.114	30.09
704.40	12.43	8.770	4.365	4.703	2.341	33.23
		10.019	3.116	5.373	1.671	23.72
Average		9.324	3.811	5.000	2.042	29.01
		9.259	4.083	5.584	2.463	30.60
804.40	11.06	10.263	3.079	6.190	1.857	23.08
		9.106	4.236	5.492	2.555	31.75
Average		9.543	3.799	5.755	2.292	28.48
		9.292	4.218	6.236	2.831	31.22
906.60	9.40	9.148	4.362	6.139	2.927	32.29
		9.081	4.429	6.094	2.973	32.78
Average		9.174	4.336	6.156	2.910	32.09
		9.552	4.102	7.100	3.049	30.04
1015.00	8.97	9.175	4.479	6.820	3.329	32.80
		8.913	4.741	6.625	3.537	34.72
Average		9.213	4.441	6.848	3.305	32.52

material showed a more rapid increase than the amount of bound water per gram of dry material, but both showed continuous increases until a hydration of 1015 per cent. was reached. This represents nearly the maximum hydration for this material. At this hydration 3.305 grams of water (32.52 per cent.) were bound per gram of dry kelp. These values indicate that the proportion of dry material in a colloidal system is a factor which greatly affects the weight of water bound per gram of dry material.

FREUNDLICH (**4**) has proposed a general parabolic equation for an adsorption reaction. The curve for bound water given in figure 1 appears to approximate a parabola. When these same values are converted into logarithms and graphically plotted, the points tend to fall on a straight line as shown in figure 2. The values of any parabolic equation plotted as

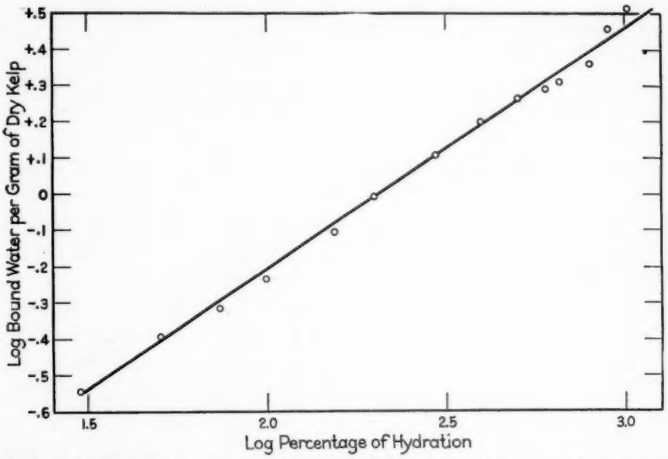

FIG. 2. Bound water and percentage of hydration values of figure 1 expressed logarithmically.

logarithms give a straight line curve. This indicates that water binding in kelp stipe may be considered as an adsorption phenomenon.

The same general type of curve for the relation between the concentration of dry material in a colloidal system and bound water was obtained in these determinations as has been reported recently by BRIGGS (**2**), JONES and GORTNER (**11**), and NEWTON and MARTIN (**22**). These studies, however, except for the calorimetric data of BRIGGS, were all made over a much narrower range of water content than were the results reported here. It is necessary to have at hand data covering the whole range of hydration of a colloid in order to gain a complete idea of the relationship between the dry material and the condition of the water in the system. This is espe-

cially true if comparisons are to be made with the colloidal systems of living cells where the range in possible moisture contents is very wide.

When bound water values for different systems of the same concentration of dry material are compared, the values usually vary considerably. This difference in the absolute values determined for bound water may be due to the dissimilarity in the nature of the colloidal systems used, or to the fact that different methods were used in obtaining the values. That the type of colloidal system is a factor in affecting the degree of water binding has been shown by several of the previous investigators. This study reports comparatively high bound water values for kelp stipe. NEWTON and MARTIN (**22**) and BRIGGS (**2**) both obtained their highest values for bound water with agar, a carbohydrate material similar in composition to kelp stipe, which may be taken as further evidence that kelp stipe has a relatively high water-binding capacity.

SAYRE (**26**) made a comparison of three methods of measuring bound water in an 18.6 per cent. gum arabic solution. He found excellent agreement in the mean values for bound water as determined by the three methods. However, when the same methods were applied on the same sample of expressed sap from corn tissues, the results were not in such good agreement. BRIGGS (**2**) determined the relative vapor pressure-water content curve for a purified colloid and then compared values for bound water obtained by different methods with points on this curve. He found that the cryoscopic method of NEWTON and GORTNER (**21**) and the vapor pressure method of HILL (**9**) yielded to a satisfactory explanation in terms of this curve, but he could not explain the different results obtained when the calorimeter method was used. GORTNER (**7**) emphasizes the fact that there is no sharp division line between bound and free water in a colloidal system. He suggests that this might be the reason why results obtained by different methods may vary so widely, since one method may measure the water at one degree of activity and another method at a slightly different degree of activity.

Attempts have been made to discover whether any correlations exist between bound water and other properties of living tissues. As previously noted, a number of investigators have observed a positive correlation between bound water, hydrophilic colloids, and cold resistance. Other factors, as sugar and moisture contents of the tissue and osmotic pressure and total solids of the tissue fluids, have also been observed by some of these workers to be related to bound water.

NEWTON and MARTIN (**22**) reported a high positive correlation between percentage bound water, concentration of solids, and osmotic pressure in the tissue fluids of some thirteen species of grasses of different degrees of drought resistance.

GORTNER and RUDE (**8**) calculated the coefficients of correlation for all the various combinations possible given in the data of MEYER (**15**) on the physico-chemical properties of plant saps from some fifty species of mesophytic plants of Ohio. The properties which had been determined were percentage water and yield of sap from the leaves; and of the total solids, osmotic value and percentage bound water of the expressed leaf saps. They did not find high coefficients of correlation between bound water and any of the other factors.

The recent work of MEYER (**17**) on cold resistance in the leaves of the pitch pine shows a positive correlation between the total hydration of the tissues and the amounts of both bound and free water per gram of dry material. These data are in accord with the results reported here concerning the relation of bound water to the hydration of the tissues of kelp.

The ranges of hydration of the kelp stipe as used in these experiments correspond in general to the ranges of hydration of such organic colloids as those found in living protoplasm, cell walls, etc. A hydration of 1015 per cent., the maximum attained in these determinations, represents approximately a 91 per cent. water content. The hydration of colloids occurring in plant cells may vary over a wide range but does not greatly exceed the range studied for kelp stipe in this investigation. The data obtained, therefore, may be of significance with regard to the relative amounts of bound and free water in the colloidal systems occurring in plant cells.

Summary

1. Determinations were made of the amounts of bound and free water in the stipe of the Elk kelp, *Pelagophycus porra* (Leman) Setchell, at hydrations varying from 30 to 1015 per cent. The degree of hydration is expressed as the percentage of water absorbed by the dry kelp.

2. The calorimeter or heat-of-fusion method was used in determining the amounts of bound and free water. The material was cooled to –20° C. and determinations made of the amount of water which had frozen. It is the assumption of this method that at this temperature all of the free water will be converted into ice and that the free-bound water equilibrium is not altered by the freezing.

3. The data show that the amount of imbibed water bound per gram of dry kelp increases with an increase in hydration, but not proportionately, so that the percentage of imbibed water bound decreases with increased hydration. At the lowest hydration of 30 per cent., 0.283 gram of water (95.83 per cent.) was bound per gram of dry material. As the hydration was increased the amounts of both bound and free water per gram of dry material showed consistent increases, the bound water at first increasing more rapidly than the free water, until a hydration of 156.6 per cent. was

reached. Here the amounts of bound and free water were approximately equal. Above this hydration the amount of free water per gram of dry material showed a more rapid increase than the amount of bound water per gram of dry material, but both showed continuous increases until a hydration of 1015 per cent. was reached. This represents nearly the maximum hydration for this material. At this hydration 3.305 grams of water (32.52 per cent.) were bound per gram of dry kelp. These values indicate that the proportion of dry material in a colloidal system is a factor which greatly affects the weight of water bound per gram of dry material.

4. The same general type of curve for the relation between the concentration of dry matter in a colloidal system and bound water was obtained in these determinations as has been reported recently by several other investigators for other kinds of colloidal systems, although in general they worked with a much narrower range of hydration.

5. The ranges of hydration of the kelp stipe as used in these experiments correspond closely to the ranges of hydration of such organic colloids as those found in living protoplasm, cell walls, etc. The data obtained, therefore, may be of significance with regard to the relative amounts of bound and free water in such systems.

The writer desires to express appreciation to Dr. B. S. MEYER of the Department of Botany, who suggested this problem and gave many helpful suggestions and criticisms during the progress of this investigation and the preparation of this paper.

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GRADIENT COMPOSITION OF ROSE SHOOTS FROM TIP TO BASE[1]

H. B. Tukey and E. L. Green

(with six figures)

Introduction

In the commercial propagation of plants by means of stem cuttings, it is customary to remove a shoot from the plant and divide it into sections, or "cuttings," which are then placed in the rooting medium. It is common knowledge that the tip cutting from a shoot is less woody than one taken from the middle portion, and that a cutting from the basal portion is in turn still more woody, indicating that the structure and degree of differentiation vary from tip to base.

Moreover, the behavior of cuttings, as regards their rooting responses, varies with the degree of "hardness." Schrader (3) has shown for the tomato, and Zimmerman (4) for *Weigela* and the American pillar rose, that when shoots are cut into segments, there may be a gradient in rooting response from tip to base of one nature or another, depending upon the nature of the material. It is the purpose of this paper to show the gradient in chemical composition throughout the length of rose shoots used for propagation by cuttings. In addition, a comparison is made between plants grown with and without an abundant nitrogen supply.

Gradient in composition from tip to base

Materials and methods

One-year-old, field grown, *Rosa multiflora* (Thurnb.) plants propagated from one original plant by cuttings were placed in new, 12-inch clay pots in the greenhouse on January 23, 1931, all materials having been steam sterilized. The pots were set in shallow enameled pans, and the plants kept moist from below by maintaining a constant water level in the pans.

Six pots contained rich greenhouse soil high in nitrogen, as judged by plant growth. Six others contained quartz sand and were supplied with mineral nutrients once a week, but no nitrogen. By this means plants were grown for examination and analysis which were of contrasting appearance and composition—the one group with high nitrogen and the other with low nitrogen.

Both lots grew vigorously until the last of March, when the plants in quartz sand with no nitrogen began to slow down in growth, as evidenced by shorter internodes and yellowish foliage. The vigorous growth of these plants for 60 days, however, with no external supply of nitrogen, is interesting. The plants in rich soil were dark green at this time.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 16.

By May 4, 101 days after planting, the leaves of the plants receiving no nitrogen were yellowish green with reddish margins and the canes were stiff and yellowish green in color. By contrast, the leaves of plants receiving liberal supply of nitrogen were dark green, and the canes were deep green and flexible. At this time material was cut for chemical analysis.

Several canes approximately 100 cm. in length were taken from both the high-nitrogen and the low-nitrogen plants. The two lots were kept separate, and the canes were cut into sections 10 cm. in length and numbered consecutively from base to tip. The analysis of this material is given in table I. The material from the high-nitrogen or soil-grown plants

TABLE I

GRADIENT IN COMPOSITION OF ROSE SHOOTS FROM BASE TO TIP, GROWN WITH HIGH AND LOW NITROGEN

SAMPLE	DRY MATTER	FRESH MATERIAL		DRY MATERIAL	
		ASH	TOTAL NITROGEN	ASH	TOTAL NITROGEN
	%	%	%	%	%
D1 (base)	58.38	0.992	0.180	1.70	0.31
D2	56.90	0.938	0.221	1.65	0.39
D3	56.78	0.919	0.181	1.62	0.32
D4	56.00	0.924	0.168	1.65	0.30
D5	56.95	1.013	0.193	1.78	0.34
D6	55.68	1.188	0.206	2.17	0.37
D7	49.09	1.055	0.181	2.15	0.37
D8	55.16	1.345	0.215	2.44	0.39
D9	54.87	1.525	0.235	2.78	0.43
D10 (tip)	55.34	1.887	0.298	3.41	0.54
	PLANTS GROWN WITH HIGH NITROGEN				
L1 (base)	43.85	1.100	0.245	2.51	0.56
L2	41.00	1.070	0.278	2.61	0.68
L3	39.92	1.169	0.263	2.93	0.66
L4	39.91	1.217	0.275	3.05	0.69
L5	39.13	1.275	0.313	3.26	0.80
L6	36.90	1.335	0.302	3.62	0.82
L7	33.75	1.282	0.286	3.80	0.85
L8	32.64	1.361	0.300	4.17	0.92
L9	28.72	1.338	0.287	4.66	1.00
L10 (tip)	25.75	1.545	0.378	6.00	1.47

was numbered L1 to L10, from base to tip respectively. The material from the low-nitrogen or sand-grown plants was numbered D1 to D10, from base to tip respectively.

Leaves and petioles were removed and each lot cut into pieces 0.5 to 1 cm. in length, and placed in a covered aluminum moisture dish and dried to constant weight at 80° C., 25 mm. pressure. Total nitrogen and ash were determined by the Chemistry Department of this Station,[1] using the Kjeldahl-Arnold-Gunning method for total nitrogen.

Discussion

The data in table I need no detailed explanation. They show the general increasing gradient for moisture, ash, and total nitrogen from base to tip of rose shoots. They also show the higher moisture, ash, and total nitrogen content of the high-nitrogen plants over the low-nitrogen ones.

How much steeper the gradient becomes as it nears the tip is shown by the analyses of tips in table II, to be compared with the tip cuttings D10 and L10 in table I. Although D10 and L10 were tip cuttings, they were nevertheless 10 cm. in length, whereas samples 4 and 5 in table II were the distal 2 cm. of the shoot. It would be interesting to know the condition within still narrower limits at and near the growing point.

Figures 1–6 show the distribution and proportionate amount of starch in shoots of similar composition to those used for analyses. The relation between starch accumulation and total nitrogen content is inverse; that is, although nitrogen content is greatest nearest the tip of the shoot, the starch content is lowest.

In the comparison of shoots from plants grown in high and low-nitrogen media, the accumulation of starch is less in the high-nitrogen grown shoots.

Accumulation seems to occur first in the xylem parenchyma, next in the xylem rays, next in the perimedullary zone, next in the cortex parenchyma, next in the pith, and last abundantly in the cortex parenchyma. It is found sparingly in the phloem and rarely in the cambial zone, either fascicular or interfascicular.

In the sections showing greatest accumulation of starch, the greatest abundance is to be found in the xylem rays, perimedullary zone, and cortex parenchyma.

Chemical composition of rose shoots grown with high and low nitrogen

Materials and methods

The material used for analysis was grown and prepared for analysis as already described. The shoots from low-nitrogen plants growing in quartz sand were divided into two lots, nos. 1 and 2. The shoots from the high-nitrogen plants growing in soil were divided as nos. 3 and 6. Entire shoots were used in these analyses, with the exception of 2 cm. of succulent tips, which were cut from each lot and analyzed separately as nos. 4 and 5. The data are given in table II.

[1] We are indebted to F. J. Kokoski for these analyses.

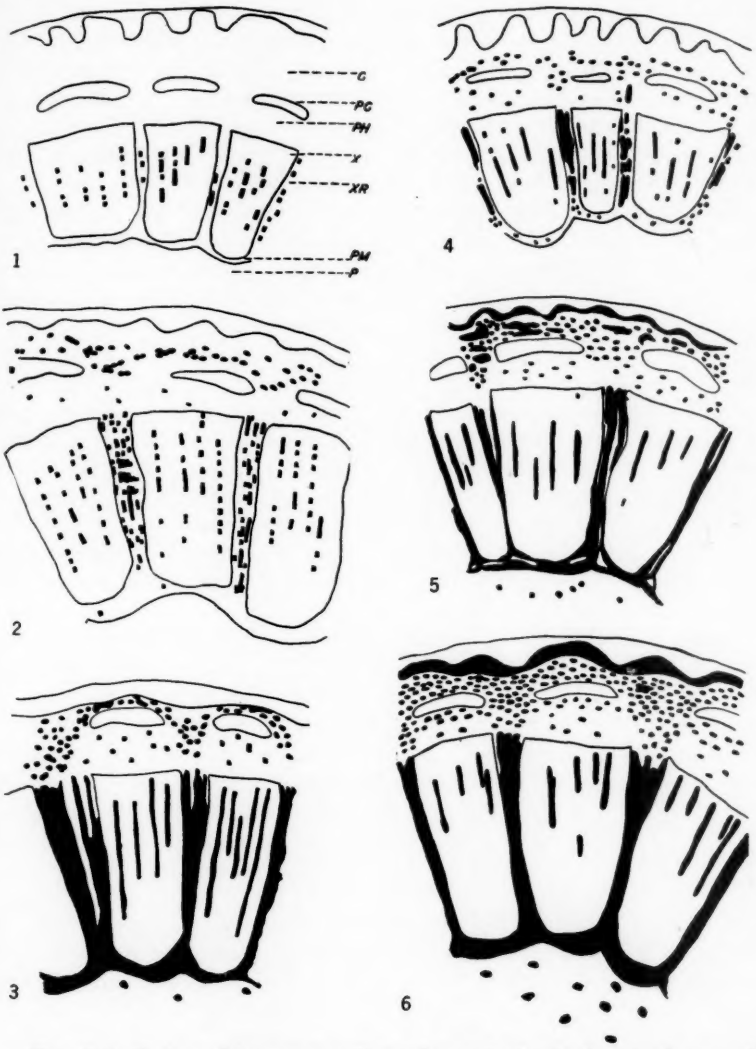
TABLE II

ROUTINE ANALYSIS OF ROSE SHOOTS GROWN IN HIGH AND LOW NITROGEN

SAMPLE	MATERIAL	DRY MATTER	FRESH MATERIAL						DRY MATERIAL				
			TOTAL SUGAR	STARCH	CRUDE FIBER	UNIDENTIFIED ALCOHOL-INSOLUBLE RESIDUE (HEMICELLULOSE)	TOTAL N	ALCOHOL INSOLUBLE N† / ALCOHOL SOLUBLE N	TOTAL SUGAR	STARCH	CRUDE FIBER	UNIDENTIFIED ALCOHOL-INSOLUBLE RESIDUE (HEMICELLULOSE)	TOTAL N
		%	%	%	%	%	%	%	%	%	%	%	%
1	Grown in low N	49.36	1.367	4.59	12.80	26.44	0.177	6.6	2.77	10.09	25.93	52.79	0.3587
2	Grown in low N	50.39	1.445	6.23	19.40	19.03	0.2051	8.2	2.87	12.36	38.50	37.76	0.4070
3	Grown in high N	38.65	1.337	1.84	14.00	15.67	0.3812	4.9	3.46	4.76	36.22	40.55	0.9860
6	Grown in high N*	37.71	1.041	1.95	13.24	15.05	0.3144	6.9	2.76	5.17	35.11	39.91	0.8340
5	Tips from plants grown in low N	28.54	.....	.....	.....	.....	0.376	.....	.....	.....	.....	.....	1.32
4	Tips from plants grown in high N	21.96	.....	.....	.....	.....	0.546	.....	.....	.....	.....	.....	2.49
7	Tips from plants grown in high N*	23.02	.....	.....	.....	.....	0.464	.....	.....	.....	.....	.....	2.01

* Two-year-old plants, intermediate between the other high- and low-nitrogen plants.

† The nitrogen in the hot 80 per cent. alcohol-insoluble residue is regarded as "protein" although the use of this term is not free from objection.



FIGS. 1–6. Sectors of transverse sections of rose shoots, showing starch accumulation: figs. 1–3, tip, middle, and basal sections respectively, from a high-nitrogen-grown plant; figs. 4–6, tip, middle, and basal sections respectively, from a low-nitrogen-grown plant.

Each lot was cut into pieces 0.5 to 1 cm. in length, and placed in a covered aluminum moisture dish and dried to constant weight at 80° C., 25 mm. pressure. The lots of succulent tips, namely, nos. 4 and 5, were then run for total nitrogen. The larger lots, nos. 1, 2, 3, and 4, were ground and again dried and weighed to determine the loss in grinding. Exactly 1 gm. of calcium carbonate was added and the whole sample extracted with hot 80 per cent. alcohol in a Soxhlet apparatus. Reducing sugars, sucrose, starch, and alcohol-soluble and alcohol-insoluble materials were determined by the methods used generally for plant materials at this Station (**2**), determining the reducing sugars by Bertrand's method (**1**) and the starch by taka-diastase followed by acid hydrolysis. Corrections were applied to allow for the loss in grinding and for the addition of the calcium carbonate. Crude fiber was determined by the official method.

Discussion

There is a high percentage of starch in the shoots from plants grown in the medium low in nitrogen (table II), and a lesser amount of starch in the shoots from plants grown in that high in nitrogen. The higher percentage of total nitrogen in shoots from plants grown in high-nitrogen soil is to be expected. Likewise the lower ratio of alcohol-insoluble nitrogen to alcohol-soluble nitrogen is to be expected, inasmuch as theoretically there should be a higher proportion of amino acids and other non-protein forms of nitrogen in actively growing parts.

These results emphasize the differences in composition between sections of a rose shoot 100 cm. in length. When shoots from plants of different growth habit are made into cuttings, there may be as much variation in composition between cuttings from the same shoot as between cuttings from different plants. Accordingly it would seem advisable to keep cuttings from a single shoot in numerical order from base to tip, and to compare the rooting habit of the sequence of cuttings as a whole rather than to make a percentage valuation as though it were a random sample.

Summary

1. Shoots of *Rosa multiflora* Thurnb., 100 cm. in length, when cut into 10-cm. sections, show a gradient of increasing moisture, ash, and total nitrogen content from base to tip, and a gradient of decreasing starch content.

2. Shoots from plants grown in a high-nitrogen medium show higher moisture, ash, and total nitrogen content, and lower starch content throughout their lengths than shoots from similar plants grown in a low-nitrogen medium.

3. The results of chemical determinations of rose shoots are presented, including dry matter, total sugar, starch, crude fiber, unidentified alcohol-insoluble residue, total nitrogen, and the ratio between alcohol-insoluble and alcohol-soluble nitrogen.

4. Sections of rose stem are reported with as much as 12.36 per cent. starch and only 0.4070 per cent. nitrogen on the dry basis; others with as much as 2.4 per cent. total nitrogen, and others with only 4.71 per cent. starch.

5. Starch accumulates first in the xylem parenchyma, next in the xylem rays, next in the perimedullary zone, next in the cortex parenchyma, next in the pith, and last in the cortex parenchyma abundantly. It is found sparingly in the phloem and rarely in the cambial zone. The greatest storage is to be found in the xylem rays, perimedullary zone, and cortex.

6. In view of the wide differences in composition, it would seem advisable in propagation experiments to keep cuttings from a single shoot in numerical order, and to compare the rooting habit of the sequence of cuttings as a whole rather than to make a percentage valuation as though it were a random sample.

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SIGNIFICANCE OF TRANSPIRATION[1]

HARRY F. CLEMENTS

Whether transpiration is beneficial or harmful to the development of plants is a much debated question. CURTIS (**11**) contends that it is almost entirely harmful with essentially no helpful contribution to the physiology of plants. MILLER (**15**) appears to agree with CURTIS when he states that "it must be admitted that its harmful effects outweigh any of the benefits that may be attributed to the process." MAXIMOV (**14**) seems to feel that transpiration is less helpful than it is harmful, suggesting that plants could adjust themselves to conditions which would prevail were transpiration non-existent. BARNES (**2**) in 1902 wrote that as plants developed on land they had to contend with this necessary evil. Others have reasoned that transpiration must be beneficial since all processes of life are of value. SHULL (**18, 20**) and others have shown the effect transpiration has on lowering temperatures.

I wish to present certain data which pertain to this subject and which may be divided into the following topics: transpiration as it reduces leaf temperatures; transpiration as it buffers leaf temperatures; transpiration as it enables rapid movement and distribution of inorganic solutes; transpiration as it aids photosynthesis; transpiration as it makes possible rapid, tall growth of plants; transpiration as a fact.

Transpiration as it reduces leaf temperatures

A tremendous amount of work has been done to determine the effect of transpiration on leaf temperatures. One procedure has been to determine the temperature of the leaf and compare it with that of the surrounding air. Thus CURTIS (**11**) points out that these obtained differences show that transpiration is of little significance since leaves usually have a higher temperature than the surrounding air. A moment's thought will show, however, that a comparison of leaf and air temperatures cannot be made since the absorption coefficient of the leaf is unlike that of the air. The capacity of the air to absorb light and radiate heat is very low, while the absorption capacity of plants is relatively high. MIX (**16**) reports that the sides of tree trunks exposed to the sun in winter with air temperatures below freezing will have temperatures as much as 39° F. above the shaded sides. HARVEY (**12**) observed that in winter a passing cloud will cause the tem-

[1] Contribution no. 36 from the Botany Department of the State College of Washington.

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perature of exposed tree bark to drop as much as 18° F. within three minutes. Thus, although comparisons of leaf and air temperatures are interesting as ecological facts, they cannot in any sense be applied to the conclusion that transpiration does little or nothing to control leaf temperature.

Leaf temperatures have also been obtained for wilted and turgid leaves. CLUM (**8**) compared the temperatures of vaselined leaves, normal leaves, and dried leaves and found small differences. However, he made no effort to determine whether or not vaseline stops transpiration, nor did he determine whether or not the coefficient of absorption is the same in dry and turgid leaves. It is significant, however, that severely wilted leaves burn very quickly if not furnished water or shade.

Another type of procedure has been to calculate the amount of heat that would exist were transpiration not a fact. BROWN and ESCOMBE (**5**) measured the total radiant energy falling on unit area of leaf surface and from this determined the rise in temperature of a leaf, recognizing the coefficient of absorption, the mass of a leaf per unit area of surface, and the specific heat of the tissue. They failed to consider adequately the amount of light reflected from the leaf. This SHULL (**19**) has done. BROWN and ESCOMBE (**5**) therefore conclude that without some means of thermal dissipation, the temperature of the leaf would rise at the rate of 35° C. per minute. Obviously thermal emissivity would prevent this rise from continuing very far, but, as I shall show, lethal temperatures would soon be reached. SHULL (**20**) has shown the possible temperature increase to vary from a few degrees in thick leaved plants to many degrees in thin leaved plants.

One preliminary experiment reported by BLACKMAN and MATTHAEI (**4**), although set up for another purpose, appears to be the only experiment on record which demonstrates the influence of transpiration on leaf temperature in which living leaves were used. A leaf was placed inside a very small glass cage. The petiole of the leaf was dipped into water so that the leaf would not wilt. The whole container was closed so that the only way that heat could be lost would be by emission from the glass. When this set-up was placed in bright sunlight, the temperature of the leaf rose quickly to 52° C., at which time the experiment was discontinued because brown spots were appearing and the leaf was being killed. Careful examination of this experiment will show that, although the leaf was allowed to transpire, transpiration was really eliminated so far as temperature determinations were concerned. As the leaf absorbed radiant energy, partially reduced in intensity by the glass, the transformed energy in the form of heat vaporized water and kept the temperature of the leaf down. However, as soon as the atmosphere was saturated and as more vapor entered it, a super-saturated condition resulted, and as condensation of this vapor took place, the liberated heat offset the heat originally used in transpiration; and since transpi-

ration existed in equilibrium with condensation, the increase in temperature was the same as would have been the case had the leaf not been transpiring at all. It is significant that the leaf was killed by the increase in temperature.

It appears that transpiration plays a very prominent part in reducing the temperature of leaves to air temperatures. In succulents such as *Opuntia, Sempervirens,* etc., where transpiration activity is very low, and where almost all of the heat disposed of is done so by means of thermal emissivity, ASKENASY (**1**) reports temperatures of 43.7° to 51.2° C., approximately 20° C. above air temperature. PEARSON (**17**) found in extreme cases temperatures ranging from 10° to 20° C. above air temperatures in tissues of *Aloe* and *Euphorbia.* URSPRUNG (**22**) found that thin leaved plants in which transpiration is more active rarely exceed air temperatures by more than 5° C.

Transpiration as a temperature buffer

MAXIMOV (**14**) warns that if leaves were constantly heated to high temperatures, the plants would soon adjust themselves to this factor. There would, however, be a danger in such temperatures. Protoplasm is itself a delicately balanced colloidal system which cannot resist wide, sudden fluctuations in temperature. CLUM (**9**), in watching the continuous temperatures of leaves, reports the largest fluctuation in 15 minutes to be 5° C. In previous work (**6**) light intensities were determined by means of a black-bulb thermometer inserted in a vacuum. The readings were made in Centigrade degrees. Invariably at night the temperature obtained from this instrument was the same as that recorded on an ordinary thermometer, but during the day there was a difference of as much as 35° C. in the readings of the two instruments. The point to be emphasized here is that the smallest cloud passing across the sun's path would cause sudden and enormous changes in the readings of the black-bulb thermometer, in one case 26° in 10 minutes in the vacuum thermometer, whereas the air temperatures remained essentially uniform. The black-bulb thermometer varied as much as 30° C. in 30 minutes while the air temperature in the same period changed about 2°. Since leaf temperatures are known to follow air temperature closely, it is obvious that the transpiration intensity fluctuates according to the fluctuations in light intensity. This then is another important rôle of transpiration: it makes possible a uniform temperature of leaves and serves as a buffer which in its variation in rate absorbs the irregularities of sunlight. Thus, although MAXIMOV (**14**) believes that plants could adjust themselves to a higher temperature, it is extremely doubtful whether they could maintain a highly active metabolism and at the same time adjust themselves to an extremely variable temperature curve.

It may be pointedly remarked that no such plants appear to exist to-day. Plants such as *Opuntia* with high tissue temperatures maintain a uniform temperature curve because of their massiveness, but thin leaved plants with high internal temperatures have not been observed. This would seem to be significant.

Transpiration as it enables rapid movement and distribution of inorganic solutes from roots to leaves

CURTIS, in arguing that transpiration is a necessary evil to plants, disposes of the value of transpiration as a rapid distributor of inorganic solutes by stating that the salts move upward through phloem tissue and therefore not in the transpiration stream, which apparently moves through xylem tissue. In a previous paper (7) the logic in CURTIS'S experiments with which he attempted to prove this point has been criticized, and data were presented which are briefly reviewed here. CURTIS'S experiments were as follows: He selected twigs of plants in pairs and girdled one. The other twig was allowed to continue growth unharmed until the girdled twig was removed. Both twigs were then analyzed to determine the ash and nitrogen contents. His results invariably showed that the girdled twig has less of both materials in it than has the ungirdled twig. He concludes that the removal of a strip of bark around the twig, which breaks the continuity of the phloem, has interfered with the upward movement of the inorganic materials. This conclusion is correct, but he further concludes that this movement takes place in the phloem. This conclusion is logically unrelated to his first conclusion and also unrelated to his data, for he makes no effort to determine the amount of material which has moved through the girdle. In my experiments, one twig or cane of a pair was girdled and the other removed at the time of girdling. Analysis of these two twigs or canes demonstrated, first, the amount of material present above the girdle at the time of girdling, and second, the amount of material present above the girdle after the girdled twig or cane had grown through the experimental period. In every case a large increase in ash and nitrogen demonstrated that the movement of these materials is chiefly in the xylem. Without question a girdle interferes with the movement in the xylem, but only in some cases does it stop the movement and then only when it stops the water movement as well. Reasons for this interference appear to lie in the anatomical relations of the water conducting tubes.

Thus, contrary to CURTIS'S statement that the transpiration stream does not carry the salts, results reported elsewhere demonstrate that it does.

Transpiration as it affects speed of growth and stature of plants

Whatever the process may be by which soil solutes enter the living cells of plants, it is probably the same whether the plant is transpiring rapidly,

slowly, or not at all. What effect various rates of water loss may have on this process has never been satisfactorily determined. However, as soon as these solutes have entered the xylem they are in the transpiration stream and move upward with the water. SHULL (**10**) has pointed out that if molecules moved only by their own kinetic forces, the movement up trees would be extremely slow. He further points out, "It is the transpiration that gives rise to the mass movement of the water in the tracheal system, and in this current the ions and molecules are swept along." The advantage of transpiration here then is that it allows for a rapid distribution of mineral solutes once these have entered the plant. Furthermore, since this is true, a more rapid growth mechanism results in a greater extension of the root system with its resulting greater absorption.

MAXIMOV (**14**), in stating that plants would adjust themselves to higher temperatures if they existed, apparently forgot that in the environment of plants there are some unchanging constants. For example, were plants heated to 55°–60° C., would other factors remain in the same relation as at present? Probably not. BLACKMAN (**3**) and others have shown that when the temperature is favorable for photosynthesis, 25°–30° C., CO_2 absorption is the limiting factor. One can at once see the effect temperatures between 55° and 60° C. would have on the absorption of this important gas. The solubility of CO_2 at 15.6° C. in 100 volumes of water is 100.5 volumes; at 26.7° C., 68.6 volumes; and at 65.6° C., 11.4 volumes. Thus the absorption of the gas would be decreased about 84 per cent.; and since CO_2 absorption is already the limiting factor at 30° C., at 65° C. it would make rapid growth impossible. Furthermore, if thermal emissivity were the only means of thermal dissipation, the added 10°–20° C. of temperature would similarly retard photosynthesis. In this way the limitations of salt movement and CO_2 absorption without transpiration would give, instead of the luxuriant vegetation characteristic of regions where water is plentiful, a sparse, small, and slow growing flora characteristic of the desert.

Transpiration as a fact

Transpiration has unquestionably been a fact since plants originated. It is due to a property of water, namely, vapor tension, and as soon as terrestrial plants developed, they were influenced by it. It would indeed seem strange if today, after many years of evolution, the development of plants had not profited by one of the fundamental properties of water. Water plants do not contend with the same factors as land plants, of course, for although they may become very large, they are never farther removed from the source of water and salts than by the distance across a few cells. Land plants have not only been benefited by transpiration, as already pointed out, but they are dependent on it to keep them in proper nutritional balance after they have once become adapted to a given environment. Al-

though plants of a xeromorphic nature could utilize more water than is usually available in their native haunts, to which they are driven by the more efficient users of water, there is a limit to the amount of water any land plant can use. Plants characteristic of regions with 20 inches of rainfall and bright sunshine produce a certain yield of fruit, but when grown in regions of much rainfall and cloudy weather they develop a rank, succulent growth which fruits either not at all or only sparsely. Transpiration here serves as a controller of the internal nutritional status of the plant; for as KRAUS and KRAYBILL (**13**) have shown, the carbohydrate-nitrogen relationship can be markedly changed when water utilization is altered.

Even when transpiration must be reduced, it itself brings about profound changes within the plant to accomplish this end; for as SPOEHR (**21**) has shown, the pentose metabolism of succulents is a function of water deficit. Since succulence is of advantage to life on the desert, the process which provides for it must likewise be advantageous.

Further, plants characteristic of the temperate and arctic zones must withstand freezing temperatures through a part of the season. Plants with an over-supply of water late in the fall are invariably more susceptible to winter killing than plants growing under conditions favoring a limited supply or a liberal loss of water. Here, too, transpiration plays a part.

In the course of this discussion several beneficial functions of transpiration have been pointed out with respect to plants as we know them today: (1) It is a cooling agent. (2) It makes possible a buffered temperature curve. (3) It serves as a rapid distributor of soil solutes once they are within the xylem. (4) It enables the rapid growth of plants by (a) hastening salt distribution in the plant, and (b) cooling the leaves and thus aiding the solubility of CO_2 in plants and thereby increasing the rate of photosynthesis. (5) It serves as a stabilizer of the internal nutrition of plants with respect to both fruiting and tolerance of unfavorable conditions.

But there is no process in physiology which is not detrimental to the organism at some time. Osmosis when too active may cause splitting of various plant parts. Photosynthesis may be harmful to plants growing in infertile soils by causing accumulations of carbohydrates and ultimately bringing about the death of the plant. Respiration, too, may be harmful when too intensive. In a similar fashion, transpiration may cause the death of a plant which is so constructed as to use it. But such harmful effects are not so much due to the process itself as they are to the limiting factors which make the normal functioning of an essential process impossible.

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EFFECT OF SOFT X-RAYS ON GERMINATION OF WHEAT SEEDS

HARRIS M. BENEDICT AND H. KERSTEN

(WITH SIX FIGURES)

Introduction

The effect of x-rays on the growth of seeds has been the subject of much investigation in recent years. Mutations, such as reported by GOODSPEED (3) in the case of tobacco, and changes in the rate of growth of the plants have been the principal results. While the literature has become too extensive to quote completely here, it should be noted that SHULL and MITCHELL (7), JOHNSON (4), and others state that small doses cause an increase in the rate of growth while LALLEMAND (6), CATTELL (2), and JOHNSON (5) claim that such doses cause no increase. All agree that large doses decrease the rate of growth.

These investigators used the ordinary Coolidge tube as a source of radiation, and thus made use of the hard, or short wave-length x-rays. The writers had available a gas x-ray tube having a window which transmitted the soft or long wave-lengths, and which could be operated at a voltage too low to produce the hard radiations. With this equipment available it was decided to irradiate wheat seeds with soft x-rays and carry out some of the determinations commonly made on seedlings.

Some preliminary experiments on the effect of these x-rays on the growth of wheat seeds, previously soaked in water, showed that when large doses were administered the wheat germinated until the coleoptile was about 2 cm. long, and remained this size for about ten days, after which it died. This might indicate, among other things, that the rayed seed had difficulty in utilizing the stored food; that is, that its starch was not so readily converted into sugar as in the unirradiated control seeds, and that respiration of the sugar was diminished. It might also indicate that the absorption of water necessary for germination was reduced.

To test these possibilities, the diastatic activity, reducing sugar content, respiration rate (as determined by comparing the dry weight before and after germination), and the percentage of water at the end of the germination period were determined for seeds irradiated for various lengths of time.

Methods

The x-ray equipment consisted of a copper target gas x-ray tube operated from a full-wave Kenetron-rectified alternating current supply at 10 milliamperes and 18 peak kilovolts. The window of the tube consisted of 0.035 mm. of aluminum and 0.04 mm. of cellophane, located about 3 cm.

from the focal spot. The seeds were held in special holders with their embryo ends toward the target of the tube and 1 cm. distant from the window. This arrangement permitted a lead shutter to operate between the seeds and window as shown in figure 1. This window easily transmitted

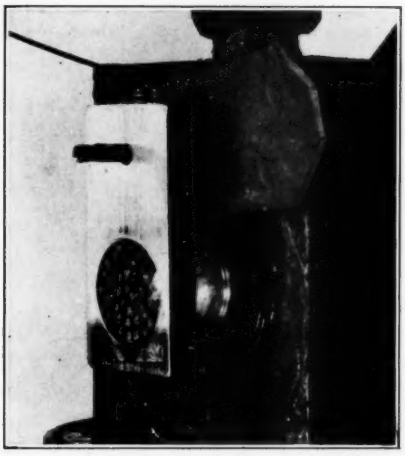

FIG. 1. X-ray tube window, shutter, and seeds in the holder.

the characteristic K_α and K_β (1.537 Å and 1.389 Å) lines of copper. Figure 2 shows a photograph of the radiation made in an oscillating rock-

K_β K_α

FIG. 2. Spectrogram of the radiation used, showing the K_β and the K_α lines of the copper target.

salt crystal spectrograph in which the two lines stand out strongly, indicating that the radiation was nearly monochromatic.

The wheat seeds (var. Purdue 352) were soaked for 12 hours in distilled water, at the end of which time they were found to contain an average of 28 per cent. water. They were then irradiated, sterilized in 10 per cent. formalin for 20 seconds, and placed between blotters in zinc trays to germinate in the dark for seven days. The trays had previously been sterilized with 0.1 per cent. mercuric chloride solution. After germina-

tion the seeds were dried for 48 hours at 40° C. and the following determinations made.

The diastatic power was determined by the Wohlgemuth iodine method. The dried seedlings were ground to a powder and extracted with distilled water (using 15 cc. per gram of powder) for three hours, with occasional stirring. Various quantities of the filtered liquid extract were added to 5 cc. of soluble starch solution and the enzyme allowed to act for three hours at 40° C.

The total reducing sugar was found by determining the amount in the enzyme extract and the amount left in the residue and adding them together. The sugar in the residue was extracted with alcohol according to the method described in the A.O.A.C. (**1**). The copper was reduced by the Munson-Walker method and determined by the volumetric permanganate method. The amount of material respired was obtained by subtracting the dry weight of the germinated seeds from the original dry weight of the seeds.

Each determination was made on 200 seeds.

Results

In figures 3–6, the results of the various determinations are plotted as functions of the time of irradiation of the seeds. The heavy vertical lines represent the limits of variation of the quantity plotted; the large circles represent the arithmetical averages of the corresponding sets of values; the numbers in the large circles represent the numbers of determinations made. For example, in figure 3, corresponding to 15 seconds, it is seen that six determinations were made. Since each determination was made on 200 seeds, there were 1200 seeds used. The values obtained vary from 15 to 25, but the arithmetical average, as shown by the position of the large circle, is 22. The small circles represent determinations when only a single value was obtained.

The results of the diastase determinations are shown in figure 3. The diastatic activity, determined by the number of grams of soluble starch decomposed per gram of dry seedlings per hour at 40° C., is plotted against the time of irradiation in seconds. In figure 4 are plotted the results of the reducing sugar content of the seedlings. The ordinates represent the milligrams of sugar present per gram of dry weight of seedlings. The respiratory rates of the seedlings are shown in figure 5. The milligrams respired per gram of original dry seed is plotted against the time of irradiation. Figure 6 shows the percentage of water in the seedlings at the end of the germination period.

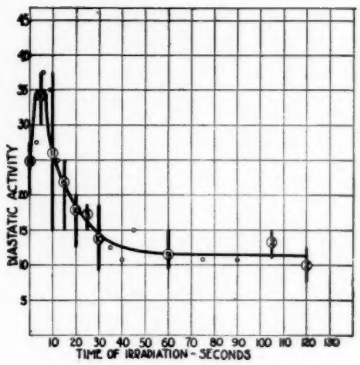

FIG. 3

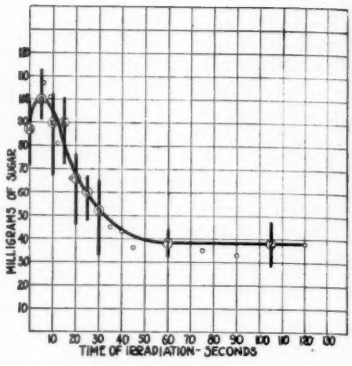

FIG. 4

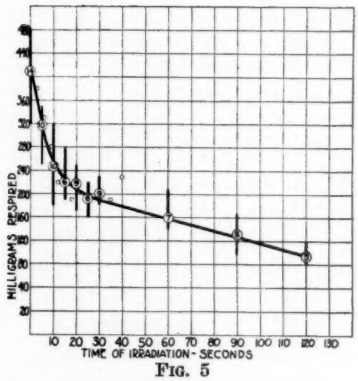

FIG. 5

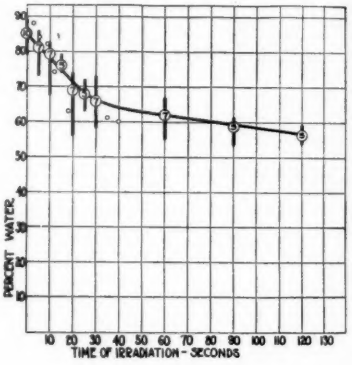

FIG. 6

FIG. 3. Diastatic activity (number of grams of soluble starch decomposed per gram of dry seedlings per hour at 40° C.) plotted against time of irradiation in seconds.

FIG. 4. Milligrams of sugar present per gram of dry weight of seedlings as a function of the time of irradiation.

FIG. 5. Milligrams respired per gram of original dry weight as a function of the time of irradiation.

FIG. 6. Percentage of water in the seedlings at the end of germination period as a function of the time of irradiation.

Discussion

The results of the diastase determinations show that there is an increase in the diastatic power during the five seconds of irradiation. That this is a true increase is shown by the fact that all the points on this line are above the highest point of the control. This does not necessarily mean that the activity of the diastase present is increased. It may mean that the ability of the seeds to produce diastase is changed by the irradiation, for the dosages of x-rays used had no effect on either wet or dry commercial taka-diastase.

The results of the sugar determinations are strikingly parallel to those of the diastase determinations. Since the function of the diastase is to convert starch to sugar, the curves for starch and sugar should be parallel and act as a check on each other.

The respiration rates, as determined by the loss of weight of seedlings while germinating, showed that the amount respired decreased with increased time of irradiation.

These three results, taken together, seem to indicate that seedlings grown from x-rayed seeds are increasingly hindered in the utilization of their stored food by prolonged irradiation.

The results of determinations of the water content of the seedlings indicate that the ability of the seeds to take in water decreases with increased time of irradiation.

Summary

1. Wheat seeds were irradiated with soft x-rays for different lengths of time, and the diastatic activity, the reducing sugar content, the respiratory rate, and the percentage of water in the seedlings were determined.

2. Wheat seedlings irradiated for five seconds show an increase both in diastatic activity and in sugar content, but if irradiated for longer time there is a decided and progressive decrease in these two substances.

3. The amount of material respired shows no increase over the controls and decreases as the time of irradiation increases. These results indicate that, under the conditions of this experiment, irradiated seeds cannot change their stored starch into sugar as readily as the controls except for very short periods of irradiation, and that even then the seeds cannot use the sugar for growth as readily as can the control seeds.

4. The percentage of water in the seedlings at the end of the germination period indicates that the irradiated seeds cannot take in water as readily as can the controls.

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BRIEF PAPERS

ABSORPTION SPECTRA OF ALPHA AND BETA CAROTENES AND LEAF XANTHOPHYLL AT ROOM AND LIQUID NITROGEN TEMPERATURES[1]

A method was developed whereby the absorption spectra of photosynthetic pigments in the solid state (glasses) were photographed with a Steinheil spectrograph. The apparatus employed was a quartz Dewar tube furnished with four plane quartz windows. The carotenoid solutions were placed in 1 mm. thick pyrex cells, which, after pre-cooling in ether at – 116° C., always were immersed in the refrigerating liquid. The cells were fastened to a metallic tube which could be raised and lowered until the cell was in the proper position. The source of continuous radiation was a 500-watt Mazda lamp operating on a 110-volt direct current. The respective sample was first photographed at room temperature, then at liquid nitrogen temperature. The carotenes were dissolved in ligroin (b. pt. 30°–35°) and the leaf xanthophyll in anhydrous diethyl ether. The error in reading band limits was between 4 and 5 per cent. The results are summarized in table I.

TABLE I

ABSORPTION BANDS OF ALPHA AND BETA CAROTENES AND LEAF XANTHOPHYLL AT LIQUID NITROGEN AND ROOM TEMPERATURES

CAROTENOID	BAND LIMITS AT LIQUID NITROGEN TEMPERATURE	BAND LIMITS AT ROOM TEMPERATURE	DECREASE IN BAND WIDTHS AT THE LOWER TEMPERATURE	MAXIMA AT LIQUID NITROGEN TEMPERATURE	MAXIMA AT ROOM TEMPERATURE	SHIFT IN MAXIMA CAUSED BY LOWERING THE TEMPERATURE
	Å	Å	Å	Å	Å	Å
Alpha carotene	5050–4900	4890–4700	40	4975	4795	180
	4700–4600	4525–4380	45	4650	4452	198
	4440–4300	.....	.....	4570	.....	.....
Beta carotene	5050–4880	4870–4665	55	4955	4767	188
	4700–4560	4535–4545	50	4630	4440	190
	4404–4260	.....	.....	4332	.....	.....
Leaf xantho-phyll	4980–4750	4850–4550	70	4865	4700	165
	4660–4460	4540–4270	70	4547	4380	167
	4345–4235	.....	.....	4290	.....	.....

The above data show (a) that absorption bands at liquid nitrogen temperature are appreciably decreased in width, (b) that bands which are difficult to recognize at room temperature become very distinct at the lower temperatures, and (c) that the maxima for carotenoids are shifted 165 to 200 Å toward the infra-red. This investigation is being extended to other photosynthetic pigments.—ELMER S. MILLER,[2] *University of Chicago.*

[1] Contribution from the George Herbert Jones Chemical Laboratory, University of Chicago.

[2] National Research Fellow in the Biological Sciences.

SOLUBLE SOLIDS IN THE WATERMELON[1]

(WITH ONE FIGURE)

In October, 1931, tests were made of the variations in percentage of soluble solids in different areas of a watermelon grown in the Snake River Valley near Payette, Idaho. This melon was a black-seeded strain of the Angeleno variety. It was cut in half and then in quarters, as shown in figure 1. Juice was squeezed from small pieces of pulp removed from each

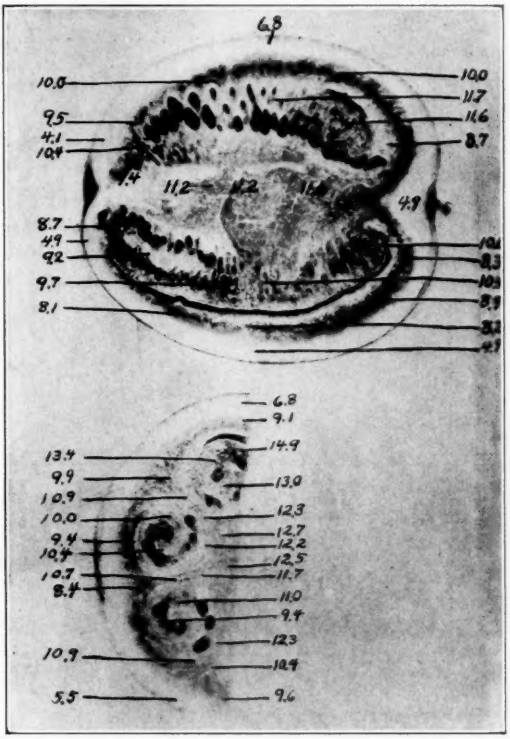

FIG. 1. Distribution of sugars in the black-seeded Angeleno watermelon.

zone and tested for soluble solids by use of the Zeiss refractometer. Most of the soluble solids in a watermelon are sugars.

As is shown in the figure, the juice in the green area just under the

[1] Contribution from the Department of Horticulture, University of Idaho, no. 107.

rind was low in soluble solids, containing 4.1 to 6.8 per cent. The red area between this green portion and the whorls holding the seeds was higher, containing about 9 or 10 per cent. in this melon. The core area ranged next with about 11 to 12 per cent., and the area around the seeds highest, with about 11 to 15 per cent. The concentration seemed higher in the tissues leading to the seeds as compared with those leading elsewhere, and also higher near the large seeds than near the small seeds toward the blossom end.—LOWELL R. TUCKER, *Department of Horticulture, University of Idaho, Moscow.*

NOTES

The Boston Meeting.—The tenth annual meeting of the American Society of Plant Physiologists at Boston, December 28–30, 1933, was a notable meeting, and reflected the ever increasing interest in the physiology of plants. In spite of the severe weather, the attendance was good, and the papers presented were in many instances of more than average merit. Some of them were of unusual value and significance. The various sessions listed 51 papers originating in 34 institutions from all parts of the United States. As growth continues, it will probably be found advisable to organize parallel programs. If rooms in close proximity were assigned for such simultaneous programs, it would be relatively easy for one to transfer from one program to another at will. The advantages would be that a stricter classification of papers could be adopted, with fewer papers on each program, and with more time for deliberate discussion and exchange of ideas. The occasional apparent lack of interest in papers is usually produced by an overloaded program, which causes even interested listeners to hesitate to take time for critical suggestions, or to present alternative views. A program which allowed about as much time for discussion as for presentation of material would obviate these difficulties, and make the programs very much more worth while to the one whose work is presented. Occasionally some one travels a thousand miles to get the unbiased criticism of the entire group, and obtains not a single suggestion because the time is too brief, the number of papers too great, for the deliberate consideration of each. This is a matter for regret, and if parallel programs would relieve the condition, it would be a very advantageous change.

Life Membership Award.—The ninth award of the CHARLES REID BARNES life membership was made at the annual dinner of the Society to Dr. JAMES BERTRAM OVERTON, for many years professor of plant physiology at the University of Wisconsin. Dr. OVERTON was born at Richmond, Michigan, December 23, 1869. His first degree, Ph.B., was obtained at the University of Michigan in 1894. Some years later, 1900–1901, he was holder of a fellowship and assisted in botany at the University of Chicago, from which institution he received the Ph.D. degree in 1901. During this period he enjoyed intimate association with Dr. COULTER, Dr. BARNES, and other members of the staff at Chicago. Following his connection at Chicago, he was a research assistant with the Carnegie Institution, and Professor of Biology at Illinois College, Jacksonville, Illinois. In 1904, he went to the University of Wisconsin as instructor in botany. Here he has continued his service, as assistant professor of botany in 1907, associate professor of

plant physiology in 1912, and professor of plant physiology since 1915. At times he has resumed his connection with the Carnegie Institution as research associate. His early research was concerned with parthenogenesis, and he has maintained his interest in cellular behavior during fertilization and reduction division. His main contributions in physiology have concerned the movements of liquids and gases in plants. His early publications on this subject dealt with sap rise in *Cyperus*. During recent years he has shared in important contributions from the Carnegie Institution on sap flow and pressure in trees, distribution of gases in trees, physiological anatomy of stems, and methods of determining the pathway of transfer of solutes and sap, especially in woody species.

Amendment of the Constitution.—A slight change was made in the constitution of the Society to permit the organization of regional sections where such action seems desirable. Sectional organizations have proved to be a great stimulus to interest in physiological research wherever they exist. With a rapidly advancing science there is great need of free exchange of ideas and mutually helpful criticism. Sectional organizations are conducive to cooperative action, and their influence on the character of the research undertaken justifies their development wherever there is sufficient interest to maintain them.

New England Section.—A significant step was taken in granting the petition of New England members of the Society for the organization of a New England Section. A large group of members representing all of the New England states signed the petition. Following the granting of the petition, the New England members held an organization meeting to elect officers and plan their future work. Dr. CARL G. DEUBER, of Yale University, is first chairman of the new section, Dr. GEORGE P. BURNS, of the University of Vermont, is vice-chairman, and LINUS H. JONES, of the Massachusetts State Agricultural College, is secretary-treasurer. Plans are already being laid for a meeting of the New England section sometime late in May, 1934. The successful development of this activity in the field of plant physiology will doubtless be of great advantage to the botanical interests of all the New England states. The future growth of the new section will be watched with great interest.

Regulations of Publication.—Important new regulations regarding publication by the Society were adopted at Boston. The regulations have received unanimous approval by the Editorial Committee, by the Executive Committee, and by the Society as a whole at its business meeting. The regulations are published here to guide authors in submitting papers for

consideration. There are three essential limitations imposed for the good of all, as follows:

(a) Free space for any one contribution is limited to 20 pages. If papers exceed this limit, authors or their institutions must find financial support for the extra space. Papers of any length may still be used if of sufficient merit, but space in excess of 20 pages must be provided for in some way by the author.

(b) Space devoted to tables and cuts is limited to 5 pages in any single contribution. Additional cuts and tables may be used, but authors or institutions must guarantee payment for any excess above a 5-page limit for a single paper.

(c) Non-members are required to pay a fee of $10.00 for each paper published in PLANT PHYSIOLOGY. In explanation of this requirement, it should be stated that PLANT PHYSIOLOGY has been open to non-members on equal terms with members during the last 8 years. In one year 27 non-member authors were accommodated. In case of a choice the editors prefer good papers, regardless of where they originate, and must continue to use only the best material available. But good papers by members have to wait unduly long for publication as a result of this liberal policy. Members are contributing toward the costs of publication, while non-members have not done so. It is merely to equalize this responsibility toward the costs of publishing that non-members are now to be required to pay a fee for the privilege of using space.

In practice this rule is to be applied as follows: Only one fee will be required when there are two or more non-member joint authors to a paper. And in case the non-member is joint author with a member, no fee will be required. In this way students will not be penalized for becoming productive before they are financially independent. The regulations under (a) and (b) apply to non-members as well as members.

These regulations are much more liberal than some that have been adopted by other societies. The Society desires to maintain PLANT PHYSIOLOGY as a most liberal journal. If conditions change so that the restrictions are unnecessary at some future time, they may be considered as a temporary expedient.

Sixth International Botanical Congress.—The following notice has been received from the Secretary of the Congress:

"The Organization Committee of the VI International Botanical Congress from various sides has been asked to change the dates of this Congress; the Committee has now decided that the Congress will meet at Amsterdam, Holland, September 2nd–7th, 1935. A first notice regarding this

Congress has been sent out to a number of addresses; for additional copies please apply to the Secretary, Dr. M. J. SIRKS, Wageningen, Holland."

Plant Constitution.—Attention is called to a publication from the press of Paul Parey, Hedemannstrasse 28–29, S.W.11, Berlin, Germany. It is entitled Pflanzliche Konstitutionslehre, by F. MERKENSCHLAGER and M. KLINKOWSKI. It deals with the internal capacities of plants to respond to conditions of their environment. It also considers the geographic origin of species, and its influence on plant constitution, particularly on the physiological direction of development. From humid regions have come species of low metabolic intensity, while from arid origins have come species with a constitutionally greater activity and productiveness.

Among the plants of humid origin described in the work are the potato, serradella, oats, buckwheat, yellow lupin, and rye. Those of more arid origin include the white mustard, sugar beet, wheat, alfalfa, and barley. The geographic migrations of these domesticated species are depicted, and an attempt made to account for the present constitution of these forms. The book is quoted by the publisher in brochure only, at RM 7.50 per copy.

British Economic Grasses.—A very useful monograph on British grasses has been prepared by SIDNEY BURR and DOROTHY M. TURNER, of the University of Leeds. It carries a foreword by STAPLEDON. About 35 varieties and species are included in the monograph. It is a taxonomic work, but should prove extremely useful to agronomists and physiologists who work with these or any other species of grass. A brief introduction gives information on vegetative and anatomical characters of grasses in general. This is followed by two keys, one based on vegetative characters, the other on anatomical characters. Following the keys are brief descriptions of the species included, with cross sections of the shoots with leaves inrolled, and of expanded leaves. Those interested in grass research will find it a very helpful guide to the structural features in this family. More books of this type are needed, for both wild and cultivated species in other families. The work is published by Edward Arnold and Co., but is distributed in the United States by Longmans Green and Co., New York, at a price of $3.75 per copy.